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Full Name of Author — Nom complet de l'auteur

Linda May Hall

Date of Birth — Date de naissance

Dec 14 1952

Country of Birth — Lieu de naissance

Canada

Permanent Address — Résidence fixe

Box 2523
Taber, Alta
Canada T0T 0G0

Title of Thesis — Titre de la thèse

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Name of Supervisor — Nom du directeur de thèse

John Hoddinott

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SOME MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF *PHASEOLUS VULGARIS* L. TO SIMULATED SUN AND SHADE LIGHT CONDITIONS

by

Linda M. Hall

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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Box 2523

Taber Alberta

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled SOME MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF *PHASEOLUS VULGARIS* L. TO SIMULATED SUN AND SHADE LIGHT CONDITIONS submitted by Linda M. Hall in partial fulfilment of the requirements for the degree of Master of Science in Plant Physiology.

John Hodgkinett
.....
Supervisor

.....

.....

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Abstract

Seedlings of *Phaseolus vulgaris* L. var. Black Valentine were grown in two light conditions of equal irradiances but different ratios of red and far red light in order to simulate the light quality of natural sun and subcanopy shade conditions and certain morphological and physiological responses were compared.

'Sun' and 'shade' plants showed different morphological responses, with 'shade' plants having less dense leaves, less chlorophyll on a fresh weight basis, greater proportions of shoot than root and higher Relative Growth Rates and Unit Leaf Rates. The 'shade' plants were photosynthetically more efficient than the 'sun' plants under these conditions.

Endogenous concentrations of IAA, measured using the indole-α-pyrone physical assay and the Meudt-Bennet bioassay showed that 'shade' plants had higher concentrations of IAA than the 'sun' plants in the first internodes, young primary leaves and young roots.

IAA oxidase isoyzmes were separated by disc electrophoresis and located with an IAA specific stain. 'Sun' plants had different banding patterns and generally fewer bands in the root and primary leaf tissues than the 'shade' plants. The implications of phytochrome involvement in enzyme synthesis are discussed.

There was a good correlation between the IAA concentration and plant morphology and higher levels of IAA

in the faster growing 'shade' plants. Also, there were generally lower levels of IAA in tissues which had higher numbers of IAA oxidase bands. The morphological, hormonal and enzyme changes due to alterations in phytochrome photoequilibrium observed are manifestations of the plant's response to subcanopy shading and sun light.

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1. Introduction

Plants exist in complex, multifaceted environments. They respond to the environment in a plastic fashion, that is, with quantitative and qualitative changes in morphology and physiology, which enables them to adapt to the environment. Such responses take place at many levels including changes in: rate of photosynthesis, the proportioning of carbon assimilates, uptake and transpiration of water, changing the morphology of the plant, endogenous concentrations of growth promotors and inhibitors, the type and activities of enzymes synthesized, membrane permeability and metabolic rate. The present study was initiated to study the effects of one environmental factor, shading by a canopy, on *Phaseolus vulgaris* seedlings.

The research presented here is comprised of a review of the relevant literature followed by three studies examining different morphological and physiological responses of *Phaseolus vulgaris* to light conditions of equal irradiance but with altered zeta ratios which simulated sun and shade conditions.

The first is a comparison of morphological responses of plants growing in the two light treatments during an early development period. Regression analysis techniques are used to determine differences in growth kinetics.

The second is an attempt to determine whether these light conditions have an effect on endogenous IAA

concentrations. The roots, first internodes and leaves are assayed for IAA using the Meudt-Bennett bioassay and the indole-α-pyrone assay.

In the third study, the IAA oxidase isozyme band patterns were examined using polyacrylamide gel electrophoresis in the same tissues over the same developmental time period used in the study of the IAA levels. Each of these studies include the introduction, methods, results and conclusions. These are followed by a general discussion of the relationship between the studies and the way in which the whole plant responds to simulated sun and shade conditions.

2. Literature Review

2.1 Light and Plant Growth

Light, the energy source for virtually all biological processes, is also one of the most important stimuli mediating plant growth and development.

The absorption of light by a biological system ia a quantum phenomena, and therefore, a proper description of the light absorbed during a reaction should contain the number of photons per second per unit wavelength interval (Seliger and McElroy, 1965). Light for plant growth varies in irradiance (the level of energy) and the distribution of that energy into various wavelengths. Physiologically active radiations range from 200 to 800 nm in wavelength, with light energies from 5.0 to -8.0 log $\mu W/cm^2$. Spectral energy distributions (SED) and irradiance levels are affected (from the norm of clear skies with direct and scattered radiation) by cloud, humidity, time of day and degree of sub-canopy shading (Holmes and Smith, 1977).

Shading by a vegetative canopy has two effects; a change in the SED and a decrease in light irradiance which causes a decrease in the energy available for photosynthesis. The filtering of sunlight by vegetation results in a decrease in blue and red wavelength bands, green being less affected, while far red is relatively unaffected (Holmes and Smith, 1977). Consequently, subcanopy

shade light has a lower ratio of red/far red light than sunlight. Light irradiance may be decreased from approximately 1400 μE/m²/sec (full sunlight) to approximately 100 μE/m²/sec (beneath a wheat canopy, as reported by Holmes and Smith, 1977).

2.1.1 Shading Effects on the Phytochrome Photoequilibrium

Phytochrome is a water soluble chromoprotein found in plant cells, and it is usually associated with the plasmalemma, etioplast and chloroplast membranes (de Greef *et al*, 1976). Phytochrome exists in two major isomeric forms, Pr and Pfr, both of which absorb light. Pr has an absorption maximum at 660 nm in the red and Pfr at 730 nm in the far red region of the spectrum. It is known that these two forms are photointerconvertible, but the actual mechanism of the photoconversion is controversial and beyond the scope of this study (Schafer *et al*, 1975; Smith, 1981). It is also known that an equilibrium is established between the two forms in multiwavelength light. This photoequilibrium has been measured in various light conditions by extracting and spectrophotometrically assaying the relative amounts of Pr and Pfr in etiolated tissue exposed to those light conditions (Smith and Holmes, 1977). The relative amounts of Pr and Pfr have been expressed as the Pr/Pfr or Pfr/Pr+Pfr ratio. These ratios have been shown to change depending on the relative energies of red and far red light (Smith and Holmes, 1977). The photoequilibrium can

be estimated by measuring the SED of the incident light, usually achieved by measuring the zeta ratio, where

$$\text{zeta ratio} = \frac{\mu E/m^2/sec\ 655\text{-}665\ nm}{\mu E/m^2/sec\ 725\text{-}735\ nm}$$

(Monteith, 1976).

Subcanopy shade light, which has a high proportion of far red light, has a low zeta ratio and therefore a low Pfr/Pr+Pfr ratio. In contrast, sunlight has a greater proportion of red light, and high zeta and Pfr/Pr+Pfr ratios.

The phytochrome photoequilibrium is related to the zeta ratio in a non linear fashion. Within natural light regimes, a small change in the zeta ratio may cause a relatively large change in Pfr/Pr+Pfr. It has been proposed that this provides the plant with a sensitive mechanism for the detection of small changes in the degree of shading in natural conditions (Smith and Holmes, 1977).

The phytochrome photoequilibrium has also been shown to be the mechanism by which a plant responds to long term sun and shade conditions (Holmes and Smith, 1977). It affects many major processes in the plant, including photoperiodism and photomorphogenesis (Smith, 1975).

2.1.2 Shading Effects on Photosynthesis

Subcanopy shade light, as compared to sunlight, decreases the photosynthetic rate by reducing the energy available for photosynthesis. However, because of the differences in SED, subcanopy and shade light support different rates of photosynthesis even in light of equal irradiance. There are two reasons for this. Firstly, all wavelengths of light are not used with equal efficiency. The light absorption by chlorophyll varies with the wavelength of irradiation. For example, an action spectrum for *Phaseolus vulgaris* var Red Kidney, grown in simulated sun conditions, showed the highest rates of photosynthesis at 670 nm and 630 nm and its photosynthetic rate declined rapidly above 680 nm (Balegh and Biddulph, 1979). Secondly, Myer and Graham (1978) have shown that far red wavelengths, when given in conjunction with other wavelengths, enhance the rate of photosynthesis, even though chlorophyll absorption of far red wavelengths is quite low. The mechanism for this effect (the Emerson enhancement effect) is not known, but it indicates that an SED with high levels of far red (shade conditions) would have a higher rate of photosynthesis than would be predicted by the action spectrum.

Boardman, in his 1977 review of photosynthesis in sun and shade plants, states that subcanopy light has lower irradiance levels and different SED than sun light, but does not separate the effects of these two factors on

photosynthesis. His analysis is consequently difficult to evaluate. He does state, however, that generally shade adapted species (those normally found growing beneath a canopy) are more photosynthetically efficient in low light irradiances than are sun adapted species.

Changes in the zeta ratios and light irradiances can affect the gross morphology of the plant and this may also affect the photosynthetic efficiency. This subject will be discussed in more detail in a subsequent section.

The structure of chloroplasts and their density in the cell is also affected by zeta ratios (Sawhney *et al*, 1980). Plants grown in low zeta (shade) conditions generally have fewer chloroplasts (Holmes and Smith, 1977) and these have large irregularly arranged grana (Boardman, 1977). The latter author has suggested that the changes in chloroplasts provide an effective means of interception of low levels of diffuse or scattered light.

Sun adapted species also have generally higher levels of RuBP carboxylase, and Boardman (1977) proposed that this could be partially responsible for the high light saturation points of these plants. The relative importance of gross morphology, chloroplast and enzyme changes on the photosynthetic rates of sun and shade adapted species is not known (Boardman, 1977).

Three studies have measured the rate of photosynthesis of plants grown in long term altered SED light conditions. Kausperbauer and Peasley (1973) using tobacco growing in

fluorescent light with end-of-day red or far red light treatments found that when photosynthesis was measured on an area basis, there were no significant differences between end-of-day red or end-of-day far red treatments. However on a fresh weight basis, there were greater photosynthetic rates in end-of-day far red treated plants. Hoddinott and Hall (In Press) using *Phaseolus vulgaris* Black Valentine and long term simulated sun and shade conditions found that shade conditions increased photosynthetic rates on both a leaf area and dry weight basis. McLaren and Smith (1978) in a study on *Rumex obtusifolius* grown in low and high zeta ratio conditions showed that simulated shade grown plants had lower net assimilation rates on an area and chlorophyll basis. These studies show that altered zeta ratios may effect photosynthetic rates independent of changes in irradiance levels.

2.1.3 Photomorphogenesis and Phytochrome

Photomorphogenesis has been defined by Mohr (1964) as the control exerted by light over the growth, development and differentiation of a plant independent of photosynthesis. When a seedling emerges from darkness into light, a series of developmental and biochemical changes are initiated in response to light. In *Sinapis alba* these processes include inhibition of hypocotyl elongation, unfolding of the lamina of the cotyledons, opening of the hypocotylar hook, initiation of the leaf primordia,

differentiation of primary leaves, formation of tracheid elements, differentiation of stomata, changes in the rate of cell respiration, synthesis of anthocyanin, increase in the rates of carotenoid, protochlorophyll, RNA and protein synthesis (Smith, 1975). All of these processes are involved in photomorphogenesis and have been shown to be mediated by phytochrome.

Photomorphogenic effects are observed to continue over the course of a plants's life, and these same processes determine the pattern of plant growth in response to sun and subcanopy shade light. Because of the range of effects the phytochrome photoequilibrium has on plant growth, photomorphogenesis has been studied at many different levels, from the effects on gross plant morphology to effects on hormones and individual enzyme activities.

2.1.4 Shading effects on Morphology

Both factors of subcanopy light, reduced irradiance and lower zeta ratios, have been shown to influence plant morphology (Hughes and Freeman, 1967; Holmes and Smith, 1977). Therefore, it is essential to separate these two factors experimentally. A recent study examined the effects of both light quality and light quantity on the morphology of *Chenopodium album*. It showed that altered zeta ratios affected stem elongation rates and leaf dry weight/stem dry weight ratios, but not leaf area or Specific Leaf Area. However, Specific Leaf Area was responsive to low irradiance

levels (Morgan and Smith, 1981). This suggests that light quality and light quantity affect different aspects of plant morphology in this species.

There are generally considered to be two ways in which a species responds to natural shading conditions; shade tolerance and shade avoidance (Grime, 1966). Shade tolerance occurs primarily in shade adapted species. Morphological changes which tend to increase the photosynthetic capacity of a plant, such as increased leaf area, are generally considered shade tolerance adaptations. Shade avoidance responses, found in species which generally grow among herbaceous plants, may serve to overtop the shading canopy by increased stem elongation.

In a comparison of the response of sun and shade adapted species to altered zeta ratios, Holmes and Smith (1977) reported that there was a correlative relationship between species habitat and the response to simulated sun and shade conditions. All species showed some response to simulated shade, including a reduced chlorophyll content and a decrease in leaf dry weight/stem dry weight ratios, but sun adapted species showed a significantly greater increase in stem elongation rates than shade adapted species. This would indicate that sun adapted plants produce a morphology typical of shade avoidance in low zeta ratios more readily than do shade adapted plants.

In conclusion, there is evidence to suggest that morphological responses vary with light irradiance and zeta

ratio, and that different species and species adapted to different habitats have different reactions to shading.

2.1.4.1 Analysis of Growth

Because growth and photomorphogenesis are dynamic phenomena, it is desirable to describe them by the use of a method of analysis which determines growth kinetics. There are two general approaches to the analysis of growth in response to environmental conditions. The first, classical, method (Evans, 1972) utilizes large, infrequent harvests of plant material. The parameters usually measured are height, fresh and dry weights of plant parts, and leaf surface area. The data collected are a large number of measurements at a few points in time. This method gives precise mean values and estimates of population variance for comparative purposes, but it does not facilitate a detailed analysis of changes in growth parameters with time.

A second approach depends on frequent, small harvests to derive mathematical functions which express changes in growth parameters with time (Hughes and Freeman, 1967). The same growth parameters are measured as in the first method, but generally, the mean values derived have larger variances because of the smaller data set at each point in time.

There are several advantages to the use of fitted mathematical curves to express the relationship of growth to time (see, for example, Hunt, 1979). The data collected from frequent harvests is a set of observed measurements

scattered randomly about reality. A mathematical function may serve to express that relationship in a clearer and more exact manner than the original data (Hunt, 1979). Small random deviations from the overall trend are smoothed out, and the nature of such time dependent phenomena as ontogenetic drift, and changes in the relative size and importance of plant parts can more easily be observed. Comparisons of compressed data can be made more easily by considering the relationship over the time span of the experiment, rather than comparing points in time which may be of limited importance in the sequence of plant development. Differences in development rates may be more easily identified, and interpolation between observation more accurate.

There are several types of mathematical curves to which growth data have been fitted. Landsberg (1977) suggests exponential, power, hyperbol and sine curves. Richard's function has been suggested and tested by several groups (Causton *et al*, 1978; Hurd, 1977; Venus and Causton, 1979). Polynomial curves have been used by a number of authors (Elias and Causton, 1976; Nicholls and Calder, 1973; Hunt, 1979) to analyze plant growth data. The type of mathematical curve chosen should provide for economy of description (Landsberg, 1977), and have the flexibility to describe a range of relationships (Causton *et al*, 1978). The function should be based on a biologically meaningful model, so that the constants themselves have meaning (Causton *et al*, 1978).

Finally, curves should have a sound statistical basis for comparison of data (Venus and Causton, 1979).

Polynomial regression equations are very flexible (almost all linear and non linear data sets may be described by polynomial regression equations), but the constants will not always have biological meaning (Landsberg, 1977). Regression equations have a well developed statistical methodology (Draper and Smith, 1976), and they are linear from a statistical point of view because the coefficients are linearly related (Elias and Causton, 1976). This allows standard parametric techniques of analysis to be employed in estimations of variance and the comparison of data. Venus and Causton (1979) found polynomial functions to be as flexible as Richard's function and that there was rarely a statistical difference between values derived by both methods. Although polynomial constants do not have explicit biological meaning, the logarithmically transformed data, when derivatized, produce the Relative Growth Rate function used in classical growth analysis (Elias and Causton, 1976) Logarithmic transformations also reduce nonhomogeneous variance in growth data.

Polynomial regression equations have beeen employed by a number of authors and there is a large body of data available for comparative purposes.

2.1.5 Phytochrome and Hormones

Since it is commonly held that the growth of plants depends on the balance of the concentrations of growth promotors and inhibitors (Scott and Briggs, 1966), it is of particular interest to look at the effects of phytochrome on endogenous hormone levels.

Although most of the studies of light effects on hormones have been carried out using flashes of red and far red light, these studies indicate a relationship is possible between hormones and the phytochrome photoequilibrium established in long term altered zeta ratio conditions.

Gibberellins have been shown by Reid, *et al* (1968) to increase in red light in etiolated barley leaves. Subsequent work by Evans and Smith (1975) showed that this was primarily due to a change in the permeabilty of etioplast membranes, permitting the movement of gibberellins from inside to outside of the etioplasts. Increased gibberellin levels in red light have also been shown for lettuce seeds, apple seed embryos (Graebe and Roper, 1978) and Sitka spruce seeds (Taylor and Wareing, 1979). These phenomena were reversible with far red light, implying phytochrome involvement.

Cytokinin levels have been shown to be reversibly increased by red light in seeds of *Rumex obtusifolius* (Van Staden and Wareing, 1972) and Sitka spruce seeds (Taylor and Wareing, 1979) as well as leaves of *Populus* sp. (Hewett and Wareing, 1973).

Ethylene production has been shown to decrease following red light flashes (Osborne, 1978). Also, studies by Bassi and Spencer (1980), using sunflower in simulated shade SED, and Erez (1977) using *Prunus persica* in subcanopy and neutral shade found that ethylene evolution increased in shade, as compared to sun light conditions.

There is also evidence for the involvement of phytochrome in the control of abscisic acid (ABA) levels. Tucker and Mansfield (1972) using *Xanthium strumarium* and supplementary red or far red light found changes in endogenous ABA levels. However Bajracharya *et al* (1975) using mustard, found no response in ABA levels to red or far red light.

The involvement of Indole-3-acetic acid (IAA) and light quality will be discussed in the next section.

Many of the affects of phytochrome on hormone levels have been shown to be very rapid. For example, work by Evans and Smith (1975) shows lag times of 30 minutes after red light treatment, before increases in gibberellins were detected. Even though these hormone changes were rapid, it is unlikely that the primary effect of phytochrome in the cell was due to a phytochrome effect on hormone synthesis, but rather that hormones were either released from a bound state (Bandurski and Schulze, 1974) or released into the cytoplasm from the etioplasts (Evans and Smith, 1975).

Studies by de Greef *et al* (1975) on the rapid transmission of light stimulus, show that when dark grown

Phaseolus vulgaris was partially illuminated, the light stimulus was transmitted within one minute to unilluminated parts of the plant. This was shown to be far red reversible and thus mediated by phytochrome. It was postulated that this response was too rapid to be a hormone effect. The rapid transmittance of light stimulus was paralleled by changes in membrane surface potential and by large increases in NADPH and ATP (de Greef *et al*, 1976). Other studies have also shown that phytochrome affects membrane associated reactions (Smith, 1975). These changes could cause alterations in cellular pH, and enzymatic reaction rates. This would exert a major influence on cellular metabolism, including the regulation of hormone levels. This suggests that some of the changes of hormone concentrations in red and far red light are secondary, rather than primary effects of phytochrome.

2.1.5.1 Indole Acetic Acid

Indole-3-acetic acid (IAA) has been intensively studied since the 1920's and consequently much more is known about the manner in which IAA concentrations are regulated in the plant than is know about other hormones. IAA is found throughout the plant in concentrations which range from 1-100 ug per Kg fresh weight in vegetative material (Schneider and Wightmam, 1978). Higher concentrations are found in seeds and storage organs.

Endogenous IAA concentrations are highly correlated with growth rates (Schneider and Wightman, 1978), and it is generally considered that the endogenous concentration of IAA and its distribution though the plant, is critical in the coordination of development (Letham, 1978). It has been shown that, to some extent, phytochrome controls the concentrations of IAA in the plant (Schneider and Wightman, 1978).

The concentration of IAA in various tissues is a result of the balance between the synthesis of IAA, transport in and out of the tissue, enzymatic oxidation, reversible formation of IAA conjugates (bound auxins), and the concentration of inhibitors, and IAA protectors.

The major sites of of synthesis of IAA have been identified, using ^{14}C labelled precursors, as the young expanding leaves and the apices (Schneider and Wightman, 1978). Other tissues also have the ability to synthesize IAA, but to a lesser extent. Five major pathways for the biosynthesis of IAA have been suggested, originating with the amino acid tryptophan and proceeding though various intermediates. There is good evidence for these proposed pathways since many of the intermediates have been found in various species. Fletcher and Zalik (1964) reported that red and far red light affected IAA concentrations in the apical portions of *Phaseolus vulgaris*. Since the apex is a major site of IAA synthesis, this work suggests that the rate of synthesis may be affected by phytochrome.

The transport of IAA may also regulate IAA concentrations thoughout much of the plant. It is generally agreed that IAA is transported basipetally in the stem and acropetally in the root. The rate of transport of exogenously applied IAA has been shown to vary with red and far red light flashes, which implies phytochrome involvement in IAA transport (Yamaki and Fujii, 1968).

The metabolism of IAA by enzymatic degradation and conjugation also serves to regulate IAA levels. The role of IAA oxidase is discussed in section 2.1.6.1. The conjugation of IAA is an apparently reversible process which may serve to store IAA. When labelled IAA is applied to plant tissue, much of the label is quickly converted to IAA conjugates (Schneider and Wightman, 1978). Bandurski (1980) suggests that conjugates have four metabolic roles in the plant:

1. sources of IAA during seed germination
2. precursors for 'seed' auxins
3. protects IAA against enzymatic oxidation
4. provides a homeostatic system to respond to environmental stimuli.

All of these could play a role in the regulation of IAA concentrations.

Plants contain substances called auxin protectors, which cause a lag period in the oxidation of IAA. These compounds protect the substrate by being strong antioxidants and they keep tissue in a reduced state. Highest concentrations of IAA protectors are found in embryonic

tissue (Yoneda and Stonier, 1967), although the chemical nature of these substances is not known (Stonier et al, 1979).

The oxidation of IAA is also modulated by phenolics (Marigo *et al*,1979). Monophenolics tend to be cofactors, whereas diphenolics act as inhibitors of oxidation. The rate of synthesis of these compounds has been shown to be affected by phytochrome (Bottomley *et al*, 1965). Phytochrome can apparently affect the endogenous concentration of IAA by more than one mechanism. The degree to which each mechanism functions in any one plant and the relative importance of each is not presently known.

Although IAA is considered very important in plant growth regulation, there are other indole compounds found in plants including Indole-3-propionic acid, Indole-3-pyruvic acid (IPyA) and Indole-3-butyric acid which have also been shown to have biological activity (Schneider and Wightman, 1978)

2.1.5.2 IAA Analysis Methods

As IAA occurs in such small quantities in plants, bioassays have been, until recently, the only satisfactory method for quantitative analysis of IAA. However, bioassays generally tend to be inaccurate and non specific compared to physical assay; they usually respond to all growth enhancers and inhibitors and not specifically to IAA. Bioassay methods are usually chosen on the basis of their relative

specificity and the ease with which they can be performed. There are two common types of bioassays, those based on straight growth such as the *Avena* coleoptile straight growth test (Sirois, 1966) and those based on curved growth such as the Meudt-Bennett bean hypocotyl curvature test (Meudt and Bennett, 1978) or the *Avena* coleoptile curvature test. Other assays have been developed based on root growth and other IAA related phenomena.

The Meudt-Bennett bioassay is a rapid and accurate method of analysis of IAA, based on the principle that unilaterally applied auxins cause curvature in stems. Meudt and Bennett (1978) reported that it can separate the effects of auxins and gibbberellins because giberellins cause only increases in length and not curvature. The sensitivity has been reported to be comparable to the *Avena* coleoptile test and it has the added advantage of being relatively unaffected by light.

Physical assays of IAA have the advantage of being generally more specific and more accurate than bioassays. They include colorimetric methods e.g. Salkowski method (Gordon and Weber, 1951); spectrophotometric techniques which measurer derivatized (Stoessl and Venis, 1970) or underivatized (Fletcher and Zalik, 1964) IAA, or by using flame ionization detection and gas chromatography (Beyer and Morgan, 1969). Flame ionization, combined with gas chromatography, is the most accurate method of analysis of IAA (Schneider and Wightman, 1978) but requires

sophisticated laboratory equipment.

The indole-α-pyrone method (Stoessl and Venis, 1970) for IAA relies on the derivatization of IAA to indole-α-pyrone, a red-orange compound with a distinctive ultraviolet, visible and fluorescence spectrum. The amount of IAA present can be estimated from the emission of the derivation product using a scanning fluorescent spectrophotometer. Improvements to this method have been suggested by a number of authors including; Knegt and Bruinsma, (1973); Ino *et al*, (1980), Eliasson *et al*, (1976), and Bottger *et al*, (1976), and have been used successfully by Pilet *et al* (1980) and others.

2.1.6 Phytochrome Effects on Enzymes

Phytochrome has been considered the only clearly defined pigment in green plants which is able to perceive light signals from the environment and translate these signals into the metabolic reactions governing development (Schopfer, 1977).

Red and far red light has been shown to have an affect on the activity of several enzymes as reviewed by Mohr (1974) and Smith *et al* (1977). Most of the studies mentioned in these reviews were carried out using monochromatic light on a few important enzymes e.g. phenylalanine ammonia lyase, ribonuclease, nitrate reductase and RuBP carboxylase. Such studies have begun to elucidate the mechanism by which phytochrome affects development.

There are many stages during the synthesis of enzymes and the course of their action, where enzyme activity could be affected by phytochrome;

1. translation of DNA information to mRNA
2. activation of new peptides
3. transformation of stored peptides
4. release of active enzymes
5. inactivation of enzymes *via* inhibitors or protectors
6. degradation of enzymes

(Schopfer, 1977).

Factors such as changes in cellular pH must also be considered, particularily considering the relationship between phytochrome and membrane permeability (de Greef *et al*, 1976). There is strong evidence that phytochrome may have an effect on the translation of mRNA from DNA (Schopfer, 1977). However, other points of control have not been excluded, and a multiple control of enzyme activity has been proposed by Frosch *et al* (1977).

In most cases phytochrome has been shown to have a photomodulating effect on enzyme synthesis, i.e. a change in the levels of activity, (Schopfer, 1977). However, it appears that phytochrome also has a photodeterminate effect, i.e. a switching on or off of an enyzme, on peroxidase synthesis in mustard (Schopfer, 1977). Peroxidase synthesis can be affected by phytochrome during an induction period during which an inactive protein is synthesized. Subsequent to this is a realization period unaffected by red and far

red light, during which the peroxidase activity increases.

2.1.6.1 IAA Oxidase

Indole-3-acetic acid oxidase (IAA oxidase) is a hemoprotein enzyme which utilizes hydrogen peroxide as a cofactor to oxidize IAA. The enzyme is closely related to peroxidase. Several studies have reported that IAA oxidase and peroxidase are apoenzymes, two active sites residing in the same macromolecule, since, when provided with the suitable substrate, they showed both activities (Srivastava and van Huystee, 1979). In contrast, other studies (Raa, 1973) reported no IAA oxidase activity in certain peroxidases.

IAA oxidase has been postulated to be a regulator of IAA concentration and thereby to affect growth rate (Hillman and Galston, 1963; Sharma *et al*,1979). The rate of IAA oxidation is regulated not only by IAA oxidase activity and IAA concentration but also by the presence of IAA protectors and phenolic inhibitors.

IAA oxidase and peroxidase have been shown to exist as multiple isozymes (Retig and Rudich, 1971; Lee, 1971 (a),(b); Lee, 1972; Anstine *et al*, 1970) i.e. enzymes with more than one molecular form which are either encoded by different genes or modified by a reactive group. (Scandalios, 1969). Anstine *et al* (1970) has shown that for some specific isozymes of peroxidase, the appearance of a new isozyme is due to *de novo* synthesis i.e. an effect on

the translation of DNA to mRNA.

Phytochrome has been shown to affect the enzyme activity levels of both IAA oxidase (Hillman and Galston, 1964) and peroxidase (Sharma *et al*, 1979). As previously discussed, the change in activity could be due to a phytochrome influence on a number of pre- or post-transcriptional events, or due to a change in concentration of inhibitors, cofactors or protectors. Since phytochrome affects *de novo* synthesis of peroxidase, and IAA oxidase exists as a number of isozymes, it is likely that a phytochrome affect on *de novo* synthesis would become apparent by looking at the pattern of isozymes, and that this could reveal some of the mechanisms by which phytochrome affects IAA concentrations in plants.

3. The Influence of Altered Zeta Ratios on Growth Parameters

> "Last year (1906) Professor Nol made a communication to the Niederrheinischn Gesellschaft fur Naturund Heilkunde at Bonn concerning the quantity which he had named the plants **Substanzquotient**. The substanzquotient was to be obtained by determining the quantity of dry substance of a plant at equal intervals and relating each weight thus obtained to the previous one, by dividing the former by the latter. Thus the dry substance quotient gives a measure of the **Assimilationsenergie** of the plant at different periods of its life, in that it relates the assimilatory income to the existing, and increasing, working capital."
>
> H. Hackenber. 1909

3.1 Introduction

Previous work on the effects of subcanopy shade and sun light on plant morphology has shown differing and occasionally conflicting results. It has been reported that a plant's response depends on the species (Morgan and Smith, 1979), the light environment that species was adapted to (Morgan and Smith, 1979), the zeta ratio (Morgan and Smith 1978), and the coincident light irradiance (Morgan and Smith, 1981). This indicates that the morphological responses of a species in a new light regime can not be predicted from the literature; and that to compare the effects of altered zeta ratios on morphology, the light irradiances must be equal.

Plant morphology can be quantified by the measurement of the dimension and weight of plant parts. However, to describe growth, morphologial parameters must be analyzed over time so that the kinetics of their relationships can be determined.

The following parameters have previously been reported to be influenced by subcanopy shading or altered zeta ratios; chlorophyll content, (Morgan and Smith, 1978), leaf area (Kasperbauer and Peaslee, 1973), Specific Leaf Area, (Morgan and Smith, 1978) dry weight of the plant (Morgan and Smith, 1978), and root/shoot ratio (Boardman, 1977). This study is an attempt to follow these, and some additional growth parameters of *Phaseolus vulgaris* grown in simulated sun and shade conditions over time, using regression analysis techniques.

3.2 Methods

3.2.1 Growing Conditions

Seeds of *Phaseolus vulgaris* L. variety Black Valentine (Rogers Brothers, Idaho Falls, Idaho, USA) were imbibed for three days in aerated, saturated calcium sulfate solution. This solution served to maintain membrane integrity and appeared to reduce the occurence of fungal infections. The seeds were placed in the dark, on sloping trays and covered with paper towels wet with saturated calcium sulfate

solution for four days. Elongated seedlings were selected for uniform height and inserted into foam stoppers. Approximately ten of these seedlings were then inserted in the plastic lids of two liter plastic pots, containing Hoagland's solution (Hewitt, 1966), and placed into one of two Controlled Environment Chambers (Chargin Falls, Ohio). The solution was aerated constantly, replenished daily with distilled water to replace the solution lost by transpiration and evaporation, and replaced weekly. The seedlings elongated for three days before being separated and placed into individual pots.

Two Controlled Environment Chambers were used to simulate the zeta ratios found in sunlight and sub-canopy shade conditions. In the simulated sunlight ('sun') chamber Sylvania (Drumonvile, Quebec) VHO cool-white fluorescent tubes were used as a light source. In the simulated sub-canopy shade ('shade') chamber were similar tubes, plus two water cooled 1000 watt quartz-iodide lamps (General Electric, USA) surrounded by two layers of orange and one layer of blue Cinituff filters (Strand Century Ltd. Mississauga, Ontario). The photosynthetically active radiation (PAR) was measured at pot height four times weekly using a Lambda Instruments (Lincoln, Nebraska, USA) LI 190S quantum sensor. Both chambers maintained a constant 20±2° C. and 55% R.H. during the 16-8 hour day-night cycle. Both fluorescent and quartz-iodide bulbs were synchronized to turn on and off at the same times. Plants were at the

greatest distance possible from the light source, in order to reduce the changes in PAR as the plant increased in height.

The spectral energy distribution, shown in Figure 1, in the two chambers was determined using a Techtum Instruments Quantum Spectrometer (Mandel Scientific Co. Ltd., Calgary), and the zeta ratio (the ratio of quantum flux in a 10 nm band width at 660 nm and 730 nm) was calculated as described by Holmes and Smith (1977). The zeta ratio of the simulated sun chamber was 3.50 and of the simulated shade chamber, 0.62. Light irradiance levels in both chambers were maintained at 185 $\mu E/M^2/sec$ as measured between 400 and 735 nm by the Techtum Instument integrator.

3.2.2 Sampling Techniques

A large group of seedlings were grown, from which 150 seedlings were selected for uniformity and randomly assigned to a light treatment. From the group of 150 seedlings, 10 plants from each chamber were randomly selected to be used for both non-destructive measurements (from day 4 to day 15) and for the destructive measurements (on day 15). Consequently both destructive and non-destructive samples were taken from the same population. Measurments were made at the same time each day to maintain orthogonality throughout the experiment.

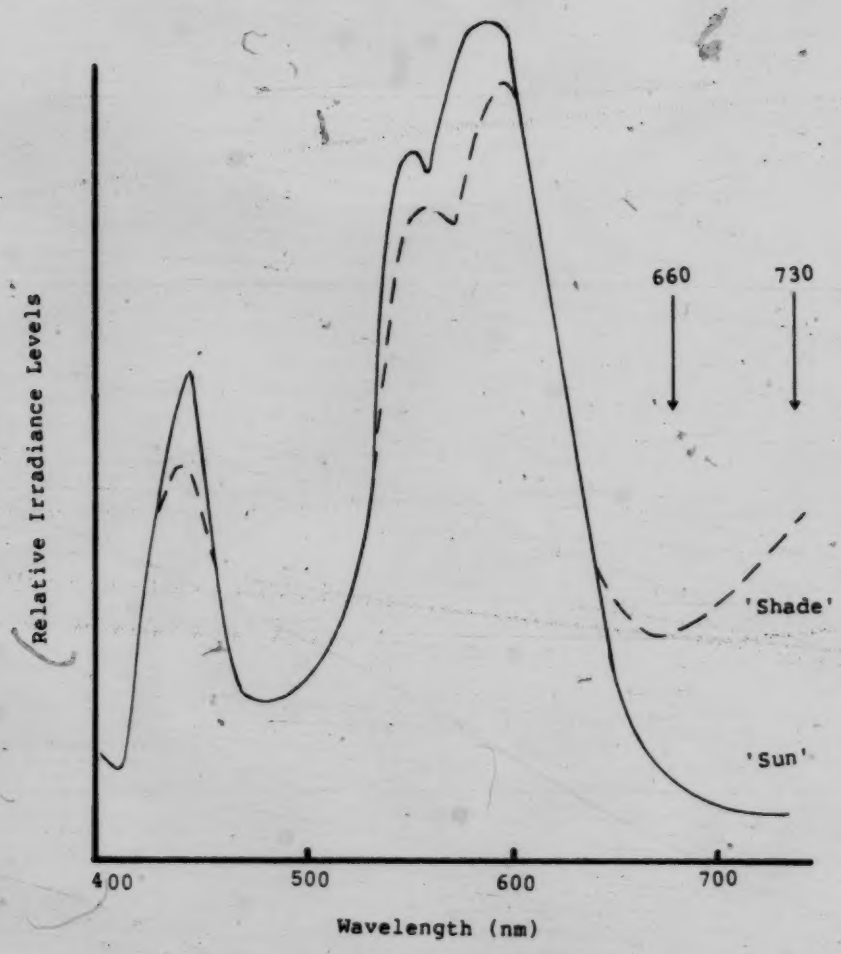


Figure 1. The spectral energy distribution of the simulated sun and shade light conditions.

3.2.2.1 Destructive Sampling

Each day from 4 to 14 days in the light, 5 plants (exclusive of the 20 plants chosen for day 15) were randomly chosen from each chamber for destructive sampling. Damaged or malformed plants were removed from the sample.

Plants were divided into; a) root b) primary leaf c) first trifoliate leaf d) second trifoliate leaf and e) internodes, and were weighed immediately to determine fresh weights. Leaf areas were determined by photocopying the leaves and then cutting out and weighing the paper equivalent of each leaf (Verbelen and de Greef, 1979). Plant parts were wrapped in foil and dried at 80° C. for 3 to 4 days and reweighed to determine dry weights.

3.2.2.2 Non-Destructive Sampling

Ten plants were randomly chosen from the large population at day 4 and from 4 to 15 days in the light, height and internode lengths were measured for individual plants.

3.2.3 Chlorophyll Analysis

The chlorophyll content of primary leaves was determined each day from 7 to 19 days in the light using the method of Hiscox and Israelstam (1979). Five leaf discs, 5 mm in diameter, were bored from primary leaves and immersed in 9 mls of dimethyl sulfoxide (DMSO) and incubated for 10 minutes at 65° C. The solution was cooled, strained and made

up to a volume of 10 ml with DMSO. The absorption was measured using a Beckman DBG Scanning Spectrophotometer (Arlington Heights, Illinois) at 645 and 663 nm against a DMSO blank. The chlorophyll a, b, and total concentrations were calculated on a fresh weight basis using the method of Arnon (1949).

3.2.4 Data Analysis

Morphological data were analyzed using the least squares method of polynomial curve fitting calculated by the Biomedical Computer Programs P-series (BMDP) program (University of California, USA).

The significance of the difference between the slopes of the regression equations for the 'sun' and 'shade' plants was tested by the method outlined by Woolf (1968).

The values for the Relative Growth Rates, Leaf Weight Ratio, Leaf Area Ratio, Specific Leaf Weight and Unit Leaf Rate were derived from regression equations using the method suggested by Hughes and Freeman (1967). The Relative Growth Rate was calculated as the slope of the regression of the natural log of the dry weight versus time. The Leaf Weight Ratio, which expresses the relationship between leaf weight and total dry weight, was calculated from regression equations as follows;

antilog(ln leaf dry weight - ln total dry weight)

Leaf Area Ratio, (cm^2/g) which expresses the relationship between leaf area and the total dry weight was calculated from regression equations as follows:

antilog(ln leaf area - ln total dry weight)

Specific Leaf Area (cm^2/g) expresses the relationship between leaf area and leaf weight. It was calculated from regression equations as follows

antilog(ln leaf area - ln leaf dry weight)

The Unit Leaf Rate ($g/cm^2/day$), a measure of photosynthetic efficiency, was calculated as follows:

$$\frac{\text{Relative Growth Rate}}{\text{antilog(ln leaf area-ln total dry weight)}}$$

from previously derived regression equations.

3.3 Results

The equations of the regressions of growth parameters versus time are presented in Table 1, along with the multiple r^2 values (coefficient of determination) which indicates the percent of the total sum of the squares of the

Parameter	a	Standard Error	b	Standard Error	Multiple r^2	t Values	Signigicance $b_1 = b_2$
ln height							
'Sun'	1.3	0.07	0.09	0.007	0.51		
'Shade'	1.4	0.05	0.08	0.004	0.69	0.96	N.S.
ln fresh weight							
'Sun'	0.21	0.19	0.13	0.01	0.52		
'Shade'	-0.17	0.11	0.19	0.01	0.84	2.44	*
ln shoot dry weight							
'Sun'	-2.06	0.12	0.09	0.01	0.58		*
'Shade'	-2.32	0.12	0.13	0.01	0.73	2.36	
ln root dry weight							
'Sun'	-4.3	0.19	0.17	0.01	0.65	2.05	*
'Shade'	-4.7	0.14	0.22	0.14	0.82		
ln leaf dry weight							
'Sun'	-4.4	0.38	0.39	0.09	0.74		
'Shade'	-5.9	0.35	0.52	0.08	0.82	6.6	***
ln total dry weight							
'Sun'	-2.01	0.13	0.11	0.01	0.65		
'Shade'	-2.24	0.11	0.25	0.01	0.77	10.1	***
ln leaf area							
'Sun'	-2.52	0.20	0.20	0.01	0.70		
'Shade'	-2.21	0.19	0.23	0.01	0.74	1.09	N.S.

* = $P < 0.05$ ** = $P < 0.01$ *** = $P < 0.001$ N.S. = not significant

Table 1. Regression equations, associated multiple r^2, standard errors and t test values for growth parameters of plants grown in simulated sun and shade light conditions.

deviation accounted for by the regression, the associated standard errors and the results of the *t* tests which determine the significance of the difference between the slopes of the regression lines of 'sun' and 'shade' plants. The graphs of the regressions for 'sun' and 'shade' treatments are shown subsequent to their descriptions.

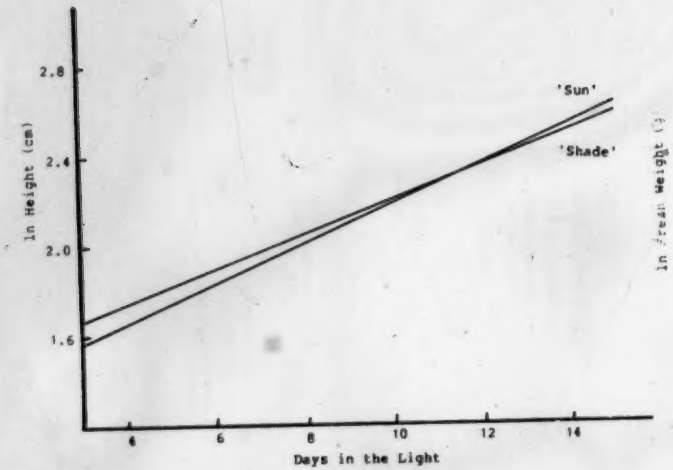
3.3.1 Height

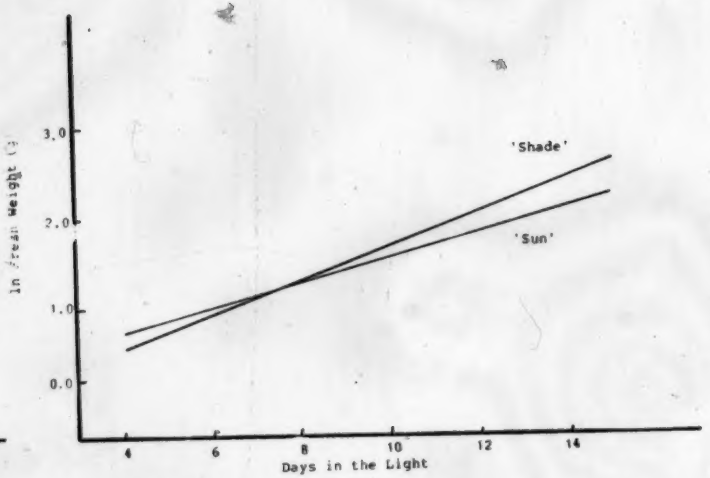
The plot of the ln of the height versus time for sun and shade plants is shown in Figure 2. Table 1 shows that there was no significant difference between the slopes of the regressions and therefore no significant difference in the rate of increase in height between the two treatments.

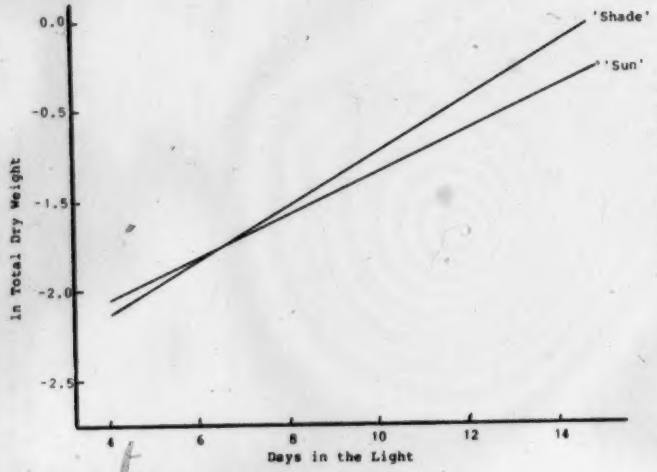
3.3.2 Fresh and Dry Weights and the Proportioning of Dry Weight

There were significantly greater fresh and dry weights accumulated by the shade plants than the 'sun' plants (Figure 2). The slopes of the regression of the ln dry weight vs time (the Relative Growth Rate) were also significantly higher in the 'shade' (Table 1). By sub-dividing the plant into shoot and root segments, it is possible to see that all the plant parts showed these differences (Figures 2 and 3) and the slopes of regression equations for roots shoots and leaves were significantly greater for the 'shade' plants (Table 1).

Figure 2. The linear regressions of ln height (cm), ln total fresh weight (g), ln total dry weight (g) and leaf dry weight (g) for plants grown in simulated sun and shade conditions between 5 and 15 days in the light.


ln Height (cm)
2.8
2.4
2.0
1.6
'Sun'
'Shade'
4
6
8
10
12
14
Days in the Light


ln Fresh Weight (g)
3.0
2.0
1.0
0.0
'Shade'
'Sun'
4
6
8
10
12
14
Days in the Light


ln Total Dry Weight
0.0
-0.5
-1.5
-2.0
-2.5
'Shade'
'Sun'
4
6
8
10
12
14
Days in the Light

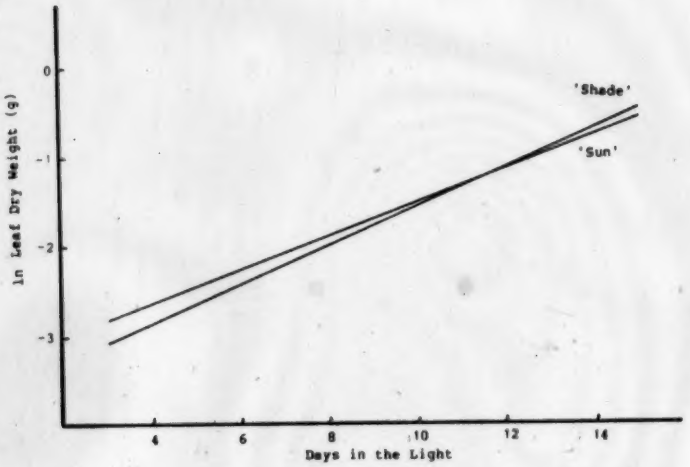
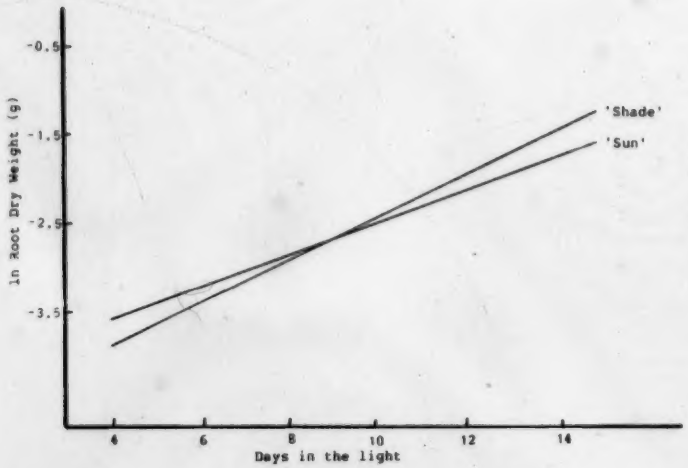
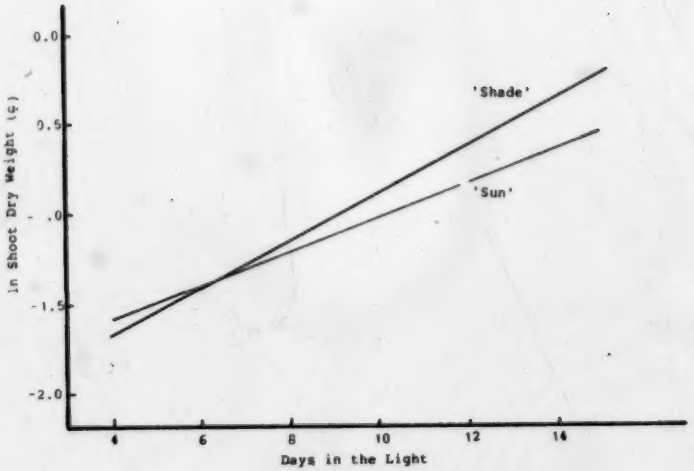
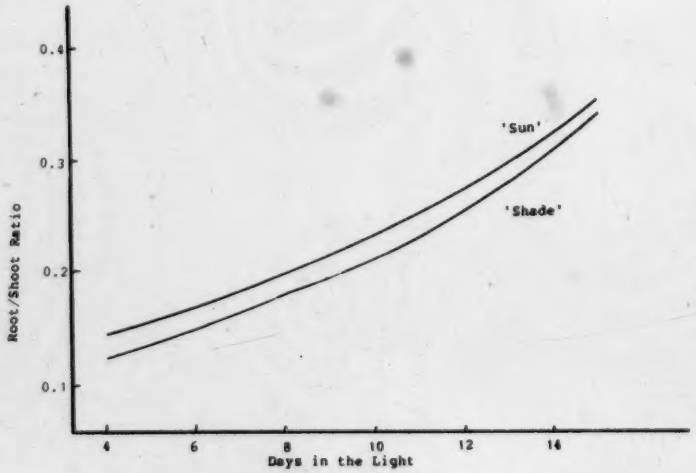
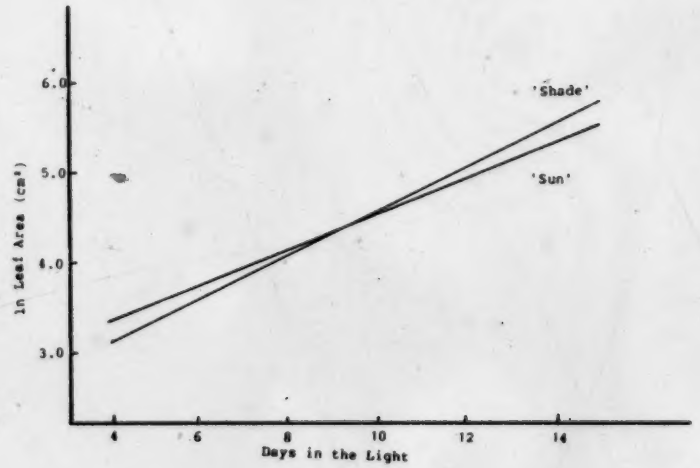

ln Leaf Dry Weight (g)
0
-1
-2
-3
'Shade'
'Sun'
4
6
8
10
12
14
Days in the Light

Figure 3. The linear regressions of ln root dry weight (g), ln shoot dry weight (g), Root/Shoot Ratio and ln leaf area (cm^2) for plants grown in simulated sun and shade light conditions between 5 and 15 days in the light.


ln Root Dry Weight (g)
-0.5
-1.5
-2.5
-3.5
'Shade'
'Sun'
4
6
8
10
12
14
Days in the light


ln Shoot Dry Weight (g)
0.0
-1.5
-2.0
'Shade'
'Sun'
4
6
8
10
12
14
Days in the Light


Root/Shoot Ratio
0.4
0.3
0.2
0.1
'Sun'
'Shade'
4
6
8
10
12
14
Days in the Light


ln Leaf Area (cm²)
6.0
5.0
4.0
3.0
'Shade'
'Sun'
4
6
8
10
12
14
Days in the Light

The relative distribution of weight into roots and shoots shows that the 'shade' plants maintained a greater proportion of the dry matter produced by photosynthesis in the shoot portion of the plant than did the 'sun' plants. This is shown by the root/shoot ratios in Figure 3.

3.3.3 Leaf Area

There were no significant differences in leaf areas (Figure 3) or the rates of increase in leaf area (slope of the regression, Table 1), between the 'sun' and 'shade' grown plants.

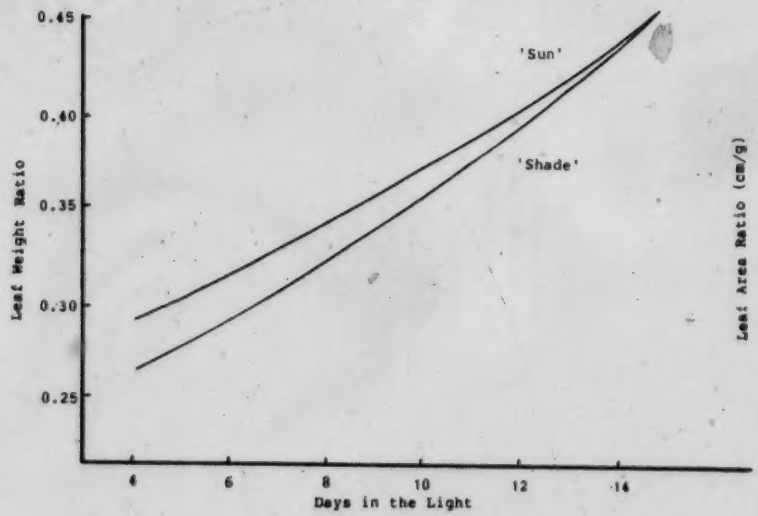
3.3.4 Leaf Weight Ratio

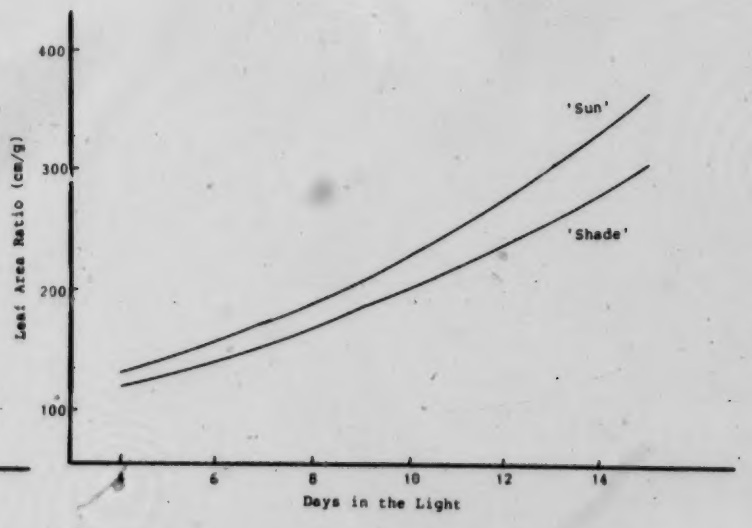
The Leaf Weight Ratio increased over time for both 'sun' and 'shade' plants. Initially, at 5 days, the 'sun' plants had a higher Leaf Weight Ratio (Figure 4) but the difference decreased with time until, at 13 days, there was no apparent difference between them.

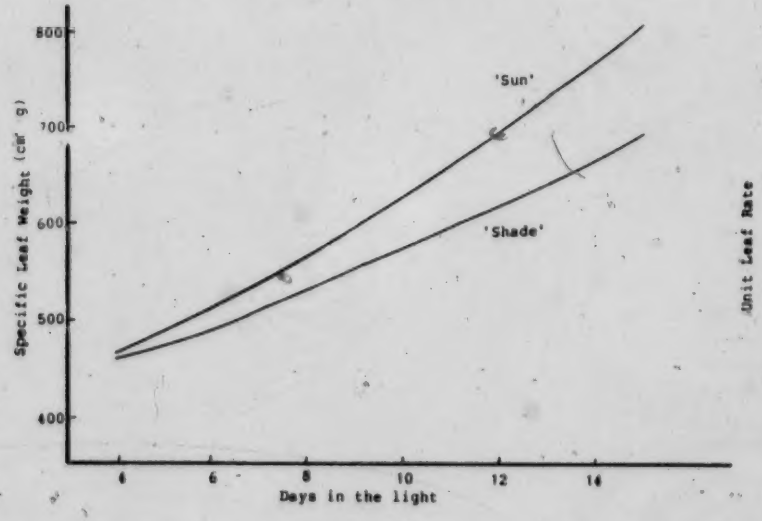
3.3.5 Leaf Area Ratio

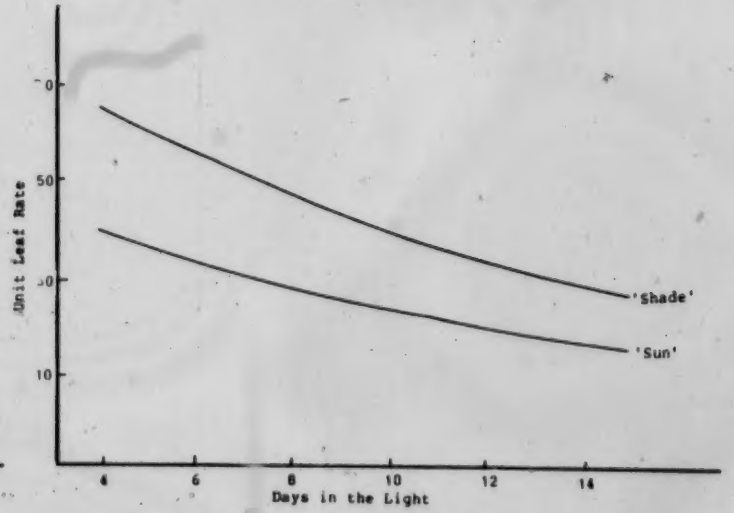
The Leaf Area Ratio also increased with time. The 'sun' plants had higher Leaf Area Ratios than the 'shade' plants throughout the developmental period studied; they had a greater proportion of leaf area compared to dry weight than the 'shade' plants (Figure 4).

Figure 4. The Leaf Weight Ratio, Leaf Area Ratio (cm^2/g), Specific Leaf Weight (cm^2/g) and Unit Leaf Rate ($cm^2/g/day$) for plants grown in simulated sun and shade conditions between 5 and 15 days in the light.


0.45
0.40
0.35
0.30
0.25
Leaf Weight Ratio
'Sun'
'Shade'
4
6
8
10
12
14
Days in the Light


400
300
200
100
Leaf Area Ratio (cm/g)
'Sun'
'Shade'
4
6
8
10
12
14
Days in the Light


800
700
600
500
400
Specific Leaf Weight (cm² g)
'Sun'
'Shade'
4
6
8
10
12
14
Days in the light


50
10
Unit Leaf Rate
'Shade'
'Sun'
4
6
8
10
12
14
Days in the Light

3.3.6 Specific Leaf Weight

The 'sun' grown plants had a greater Specific Leaf Weight than 'shade' plants thoughout the developmental period studied and the differences between the two treatments increased with time (Figure 4). This is an indication of denser leaves in the 'sun' plants.

3.3.7 Unit Leaf Rate

The Unit Leaf Rate, which is a measure of the efficiency of photosynthesis on a day weight and leaf area basis, decreased with time and was greater in the 'shade' plants than the 'sun' plants thoughout the experimental period (Figure 4).

3.3.8 Chlorophyll Content

The chlorophyll content, on a mg/g fresh weight basis was greater in the 'sun' plants than in the 'shade' plants for most of the experimental period (Figure 5). The decrease in total chorophyll in shade light appeared to be due to a decrease in both chorophyll a and b which show a similar pattern of change of concentration with time. There were no significant differences in chlorophyll a/b ratios between the two light treatments. The increased variability in chlorophyll content at the later stages of growth in both treatments was probably due to the onset of leaf senescence in the primary leaves.

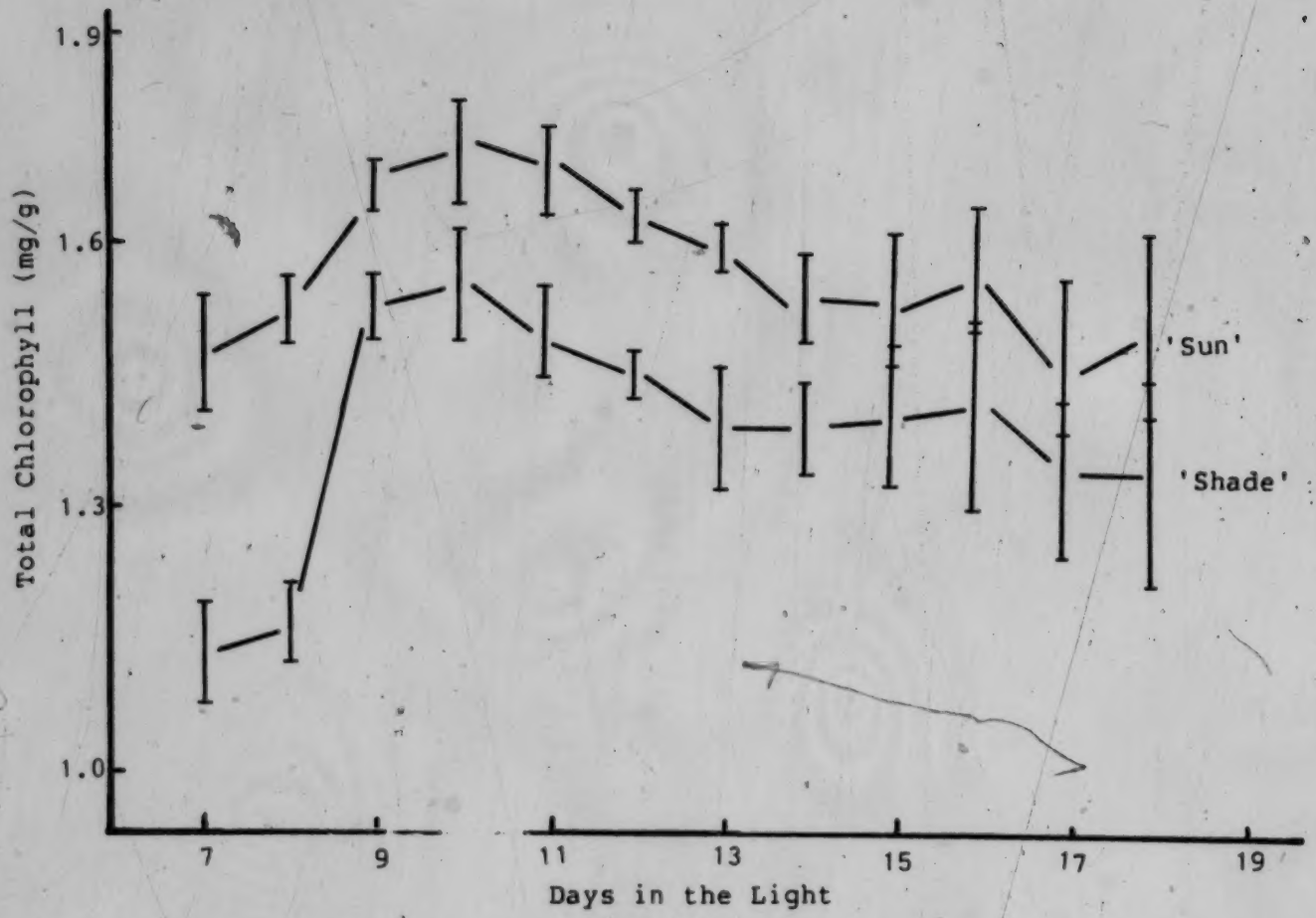


Figure 5. The total chlorophyll content (mg/g fresh weight) of plants grown in simulated sun and shade light conditions from 7 to 19 days in the light.

3.4 Discussion

To separate the morphological changes caused by altered zeta ratios from those caused by changes in the levels of irradiance, the energy available for photosynthesis must be maintained at equal levels in the simulated sun and shade light conditions. The energy available for photosythesis is difficult to measure due to the Emmerson enhancement effect, which changes that photosynthetic rate depending of the amount of far red light, and the physical limitations of light measuring instruments. The Lambda Instrument (LI) sensor, which has been used in previous studies to compare irradiance levels (Hoddinott and Bain, 1979; Morgan and Smith, 1981) was shown to be in disagreement with the Techtum instrument integrator values (Hoddinott and Hall, In Press). The LI sensor monitors different wavelengths with different efficiency and therefore should not be used to compared irradiance levels in different SEDs. It is possible that some of the previously reported effects of altered zeta ratios on morphology were due to changes in irradiance or that the effect of the different zeta ratios were masked by inaccurate irradiance balancing.

The zeta ratios in the simulated environments are more extreme than those found in natural habitats, the 'sun' being higher and the 'shade' being lower than those above and below a wheat canopy (Holmes and Smith, 1979). It was therefore expected that if a response occurred under natural conditions in the field, a similar response would have been

seen in the present experiment. A correspondence between responses of *Chenopodium album* grown in simulated and natural conditions has been previously reported (Morgan and Smith, 1981).

The present work differs from previously published work in both light conditions and plant species used. Not only were the zeta ratios and irradiance levels of the other studies different from the present study, but many authors (Morgan and Smith, 1978; 1979; 1981; Holmes and Smith 1979) gave plants pretreatment light. Initial morphological development of all plants in these studies occurred under similar light conditions (usually high zeta and irradiance, Morgan and Smith, 1981) before they were placed in simulated sun and shade light conditions, unlike this study where the plants were kept in the dark until exposed to the light treatments. This makes direct comparisons of this work and previously published work difficult.

The use of frequent small harvests and polynomial regression equations to analyze morphology allowed the kinetics of plant growth to be analyzed and compared. The logarithmically transformed data best fit linear regression equations as in previous reports by Hughes and Freeman (1967).

The present study shows how *Phaseolus vulgaris* adapts morphologically to simulated sun and shade light environments. There were no significant differences in stem elongation rates between 'sun' and 'shade' light conditions.

Previous work by Downs et al (1957) using *Phaseolus vulgaris* and far red light flashes, and work by Morgan and Smith (1979) with *Chenopodium album* in long term altered zeta ratios, has shown increased stem elongation rates in far red or shade conditions. Homes and Smith (1977) found varying degrees of change in the rates of stem elongation under simulated shade light in species adapted to different light environments. The increase in stem elongation rates in shade light is generally viewed as a shade avoidance response but this response was not evident in the present study.

Phaseolus vulgaris showed no significant differences in leaf area, in 'sun' and 'shade' light. However Leaf Area Ratios and Leaf Weight Ratios showed that, on a total dry weight basis, the 'sun' plants had a greater leaf weight and area than the 'shade' plants. McLaren and Smith (1978) using *Rumex obtrusifolius* and Holmes and Smith (1977) using *Chenopodium album* found decreases in leaf area with low zeta (shade) conditions. Kasperbauer and Peaslee (1973) using tobacco observed longer but narrower leaves in shade conditions. Leaf area has been considered very important in determining the ability of a plant to intercept enough light for photosynthesis in shade conditions, and an increase in leaf area has been suggested as a shade tolerance response by Boardman (1977).

Specific Leaf Weight was greater in 'sun' grown plants than 'shade' grown plants. This was also reported by Morgan and Smith (1978) when growing *Chenopodium album* in low zeta

conditions and by Kasperbauer and Peaslee (1973) growing tobacco in end-of-day red and far red light conditions. Specific Leaf Weight has been used as an indication of leaf thickness, with thicker leaves having less area for the same dry weight. However McLaren and Smith (1978), when they studied leaf morphologies in high and low zeta ratio conditions, found decreases in spongy mesophyll air-space volume and a loss of internal structure in low zeta (shade) conditions. They found no correlations between leaf thickness and Specific Leaf Weight. Specific Leaf Weight is therefore more correctly a measure of leaf density and a change in Specific Leaf Weight can indicate a change in leaf anatomy.

Leaf morphology has been shown by Nobel and Hartstock (1981) to be important in controlling photosynthetic rates. Increases in mesophyll surface area (such as those under 'sun' conditions) have been shown to decrease mesophyll resistance and to increase photosynthetic rates. The present study and others by Morgan and Smith (1978) and McLaren and Smith (1978), have shown that 'shade' plants have less total chlorophyll on a fresh weight basis than 'sun' plants. In addition to this, the less well developed internal stucture and the relatively less dense leaves, would suggest that plants grown in 'shade' conditions have a decreased photosynthetic ability. However, in spite of the different morphologies of the 'shade' plants, less dense leaves, lower Leaf Area Ratios and Leaf Weight Ratios, the 'shade' plants

were more photosynthetically efficient than the 'sun' plants. Both measures of photosynthetic efficiency (Relative Growth Rates and Unit Leaf Rates) were greater under 'shade' conditions. A similar response to simulated shade was observed by Morgan and Smith (1978) who found a 26% greater dry weight accumulation in *Chenopodium album* growing in low zeta conditions. The opposite response was observed by McLaren and Smith (1979) using *Rumex* which indicates that the response of increased dry weight accumulations may not be a consistent response to simulated shade light.

There were lower root/shoot ratios in the 'shade' grown plants, indicating that a greater proportions of the photosynthate stayed in the shoots rather than being translocated to the roots. The rate of translocation in similar 'sun' and 'shade' grown plants was examined by Hoddinott and Hall (In Press) and found, in proportion to the rate of photosynthesis, to be less in the 'sun' plants. This was in agreement with the observations of Morgan and Smith (1978) and Boardman (1977) who argues that shade adapted plants invest a greater proportion of their synthetic capability in the synthesis and maintanance of light harvesting machinery than do sun adapted plants.

Boardman (1977) has suggested that morphological changes are one mechanism for optimizing photosynthetic rates in subcanopy shade conditions. Increases in the rate of photosynthesis in shade light in similar plants and light conditions have been reported by Hoddinott and Hall (In

Press). A causal relationship between specific morphological changes and the increase in photosynthesis rates can not be established, however decreasing root/shoot ratios, and leaf anatomy changes are factors which may contribute to the increase in the photosynthetic rate.

4. The Concentration of IAA in Plants Grown in Simulated Sun and Shade Conditions

> "We must therefore conclude that when seedlings are freely exposed to a lateral light, some influence is transmitted from the apex to the lower part, causing the latter to bend."
>
> Darwin, 1881.

4.1 Introduction

The endogenous IAA concentration of the apical portions of dark grown *Phaseolus vulgaris* has been shown to remain higher after treatment with 7 hours of monochromatic far red light than with similar red light treatment. This implied phytochrome involvement in the maintenance of endogenous levels of IAA. Phytochrome has also been shown previously to effect the rate of transport of exogenously applied IAA (Letham, 1978) as well as the activity of IAA oxidase, an enzyme responsible for IAA degradation (Galston and Dalberg, 1954).

This study was an attempt to compare the effect of two long term light conditions on the endogenous IAA concentrations in *Phaseolus vulgaris*. The light conditions maintained equal irradiances while changing the zeta ratios to simulated sun and subcanopy shade conditions. Both a

physical assay and a bioassay were used to measure IAA concentrations in root, first internode and leaf tissue throughout an initial developmental period of 5 to 15 days in the light.

4.2 Materials and Methods

Seedling of *Phaseolus vulgaris* var Black Valentine were grown as described in Growing Conditions, section 3.2.1. At 5, 7, 9, 11 and 15 days in the light, appproximately 10 g. of root, first internode and primary leaf tissue was obtained from a uniform group of plants and weighed, frozen in liquid nitrogen and stored at -10° C. pending analysis.

4.2.1 Extraction Method

IAA was extracted and assayed using the indole-α-pyrone method (Knegt and Bruinsma, 1973) with modifications suggested by Ino *et al* ,1980; and Pilet *et al* 1979. A single extraction was made of all samples to minimize the variance due to extraction and pairs of samples were run in parallel throughout. The following steps were taken to reduce the oxidation of IAA and the conversion of IPyA to IAA during the extraction

1. all solvents and buffers were kept cold
2. Butylated Hydroxy Toluene (BHT), an antioxidant was added to all buffers and solutions
3. exposure of the plant material to light during the

extraction was minimized

4. samples were left in a dry state for minimal time periods
5. all solvents were redistilled to reduce impurities which could cause oxidation

Frozen samples were homogenized in 90% methanol and 100 mg BHT with ten ml of the methanol solution for each gram fresh weight of tissue. To the extract was added 0.4 ml of ^{14}C labelled IAA (Amersham, Oakdale, Ontario, specific activity 2.18 GBq/mmol) equivelent to 81,000 cpm and 4.90 $x10^{-9}$ g. IAA. The suspension was shaken for four hours at 5° C. before being filtered and the residue washed with 90% methanol solution. The extract was evaporated at 30° C. to an aqueous residue.

To the aqueous residue, was added 20 ml of cold 0.5 M K_2HPO_4, pH 8, containg 100 mg BHT. This was passed through a 20 x 1.0 cm column of polyvinylpyrolidine (PVP) and eluted with 0.5 M K_2HPO_4. The first 60 ml were collected and acidified to pH 3 with 2.8 M H_3PO_4. This was then partitioned three times against 30 ml of ether, washed with water containing 200 mg BHT. To the ether fraction was added 20 ml of 0.5 M K_2HPO_4, pH 8, and the ether partitioned 3 times against this phosphate buffer. The aqueous fraction was taken to pH 3.0 and partitioned three times against 30 ml of water/BHT washed ether. The ether was eluted through a 5.0 x 1.0 cm column of anhydrous Na_2SO_4 and evaporated to dryness at 30° C. The dry residue was taken up in 7 ml of

methanol and 10 mg BHT and the methanol solution divided into seven equal parts, four of which were for fluorescent assay, one for bioassay, one for yield determination and one extra.

The sample for recovery determination was dried under reduced pressure at 30° C. To the residue was added 15 ml of scintillation fluid (Econoscint, Irvine, California U.S.A.) and the radioactivity counted on a Searle Tracor Analytic Mark III liquid scintillation spectometer (Arlington Heights, Illinois, U.S.A.) for one minute. Quench was monitored using External Standard Pulse (ESP) and found to be negligible. Corrections were made for dilution and a % yield determined for each sample.

4.2.2 Physical Assay

To the fluorescent assay subsamples was added 10 mg BHT and 0.0, 0.1, 0.2 or 0.4 ml of a methanol solution of 1.75×10^{-5} M IAA standard (Sigma Chemical, St. Louis, Missouri USA). These were dried at reduced pressure at 30° C. and then refrigerated at 5° C. until use.

A Perkin-Elmer MPF-44 Fluorescence Spectrophotometer with a Xenon arc lamp and Perkin-Elmer 56 Recorder was used for spectrofluorimetric analysis. To the dry samples was added 0.2 ml of a mixture of equal volumes of cold trifloroacetic acid and acetic anhydride and 100 mg BHT. The reaction was allowed to continue for 15 minutes after which it was stopped with the addition of 3 mls 90% acetic acid.

Thirty seconds after the reaction was stopped, the fluorescent emmission was scanned from 460 to 510 nm. Excitation was at 440 nm with a half band width of 5.5 nm. The fluorescence was measured at 490 nm as suggested by Knegt and Bruinsma (1973). From the regression line of the samples containing 0, 1, 2, and 4 aliquots of IAA standards, the unknown amount of IAA was calculated. This value was corrected for background, dilution factor, extraction yield and tissue fresh weight.

4.2.3 Bioassay

IAA levels were also determined by bioassay using the method described by Meudt and Bennett (1978). Seeds of *Phaseolus vulgaris* var. Kentucky green pod (Robertson Seeds, Edmonton) were soaked for 3 days in aerated calcium sulfate solution. The seeds were placed in trays filled with vermiculite, wet with distilled water and covered by 1 cm of vermiculite. Seedlings were grown from 7 to 9 days in an irradiance of 40 $\mu E/m^2/sec$ and an 8-16, day-night cycle. Temperature and relative humidity were kept constant at 22 ±2° C. and 64% respectively. At the time of harvest plants were chosen which had first internodes which were greater than 4 cm in length and primary leaves which were beginning to expand.

Small vials (I.D. 16 mm) were filled with 10 mls of 0.1 mM NaH_2PO_4 buffer (pH 6.4). Sponges, previously washed in methanol and distilled water, were partially inserted into

the vials. Onto small discs of filter paper were pipetted 100 microliter of plant extract or IAA standards.

The first internodes were cut into 4 cm sections and placed between filter paper wet with with NaH_2PO_4 buffer (pH 6.4) until needed. Two internodes were placed on opposite sides of each vial, apical portion uppermost, between the sponge and the glass of the vial. Then between the sponge and the stem, and in immediate contact with both, was placed the dried filter paper (see Figure 6). The distance between the tips of the apical portions of the stems was measured and the vial placed in the dark for exactly 24 hours. Vials with damaged or malformed internodes were discarded (approximately 2%). After this time they were remeasured, the concentrations of IAA were determined from a standard curve and corrected for dilution factors, yield, IAA added and the fresh weight of the tissue.

4.3 Results

4.3.1 Physical Assay

Extraction recovery varied from 64 to 89%. This degree of variablity made the individual sample assessment of yields necessary.

Figure 7 shows an example of the scan obtained for a sample with 0, 1, 2 and 4 aliquots of IAA added. A change in the specrophotometer sensitivity of 3 orders of magnitude is

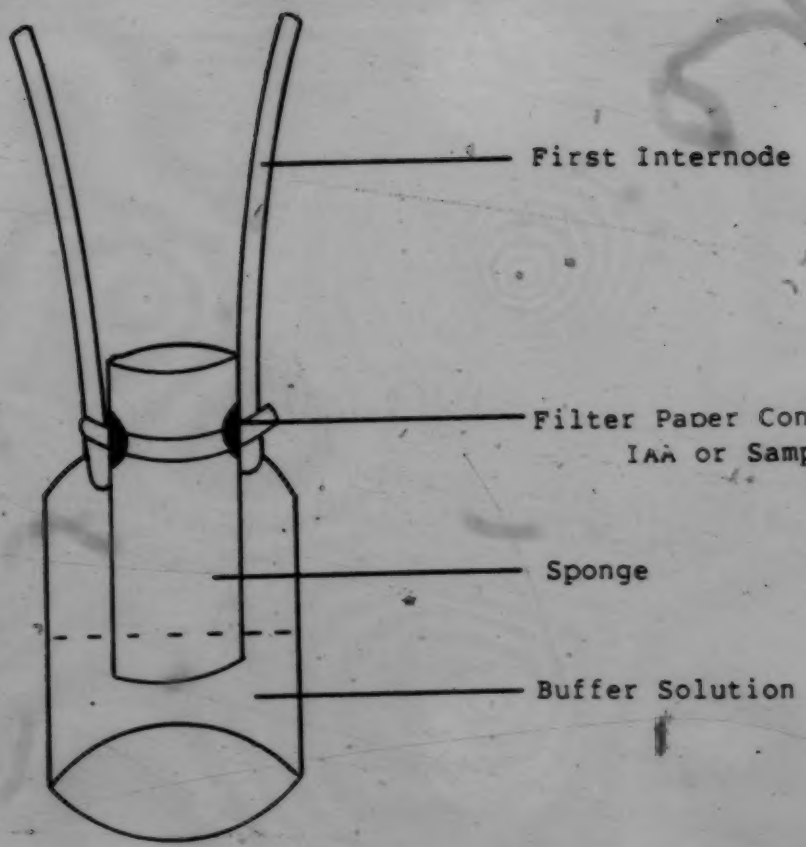


Figure 6. Diagram of the manner in which the Meudt-Bennett Bioassay was carried out.

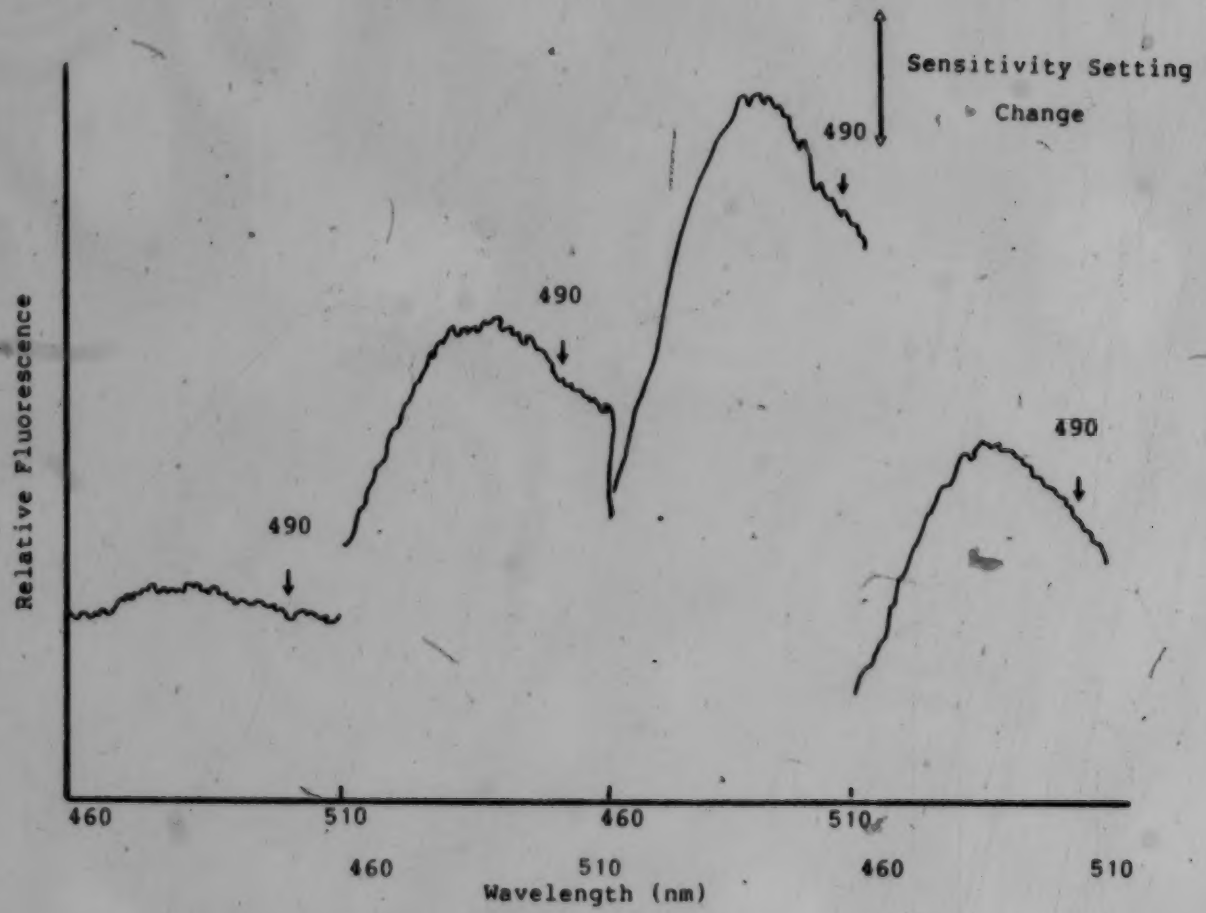


Figure 7. Typical fluorescent scan of a sample with 0, 1, 2, 4 aliquots of IAA added, using the indole-α-pyrone physical assay.

also noted. Figure 8 shows the standard curve and associated 95% confidence limits.

'Shade' grown first internode tissue had higher levels of endogenous IAA than 'sun' first internode tissue. The levels in both 'sun' and 'shade' stems decreased with age and corresponding distance from the apex. This is shown in Figure 8.

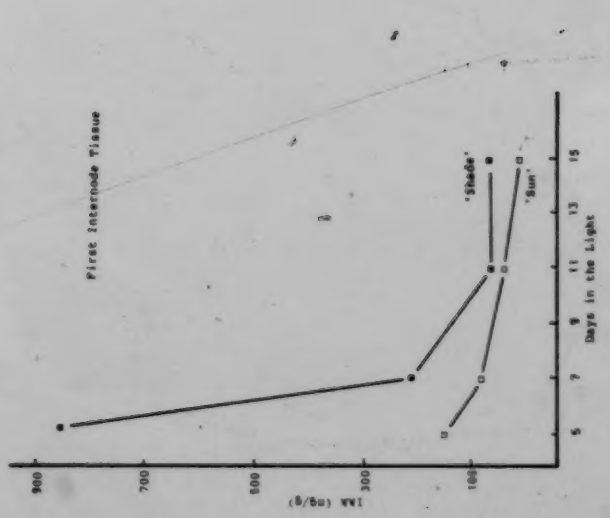
Root tissue showed lower levels of IAA than either stem or leaf tissue. Initially (5 days) there were greater levels of IAA in the 'shade' but this difference was not apparent at days 11 and 15. This relationship is show in figure 8.

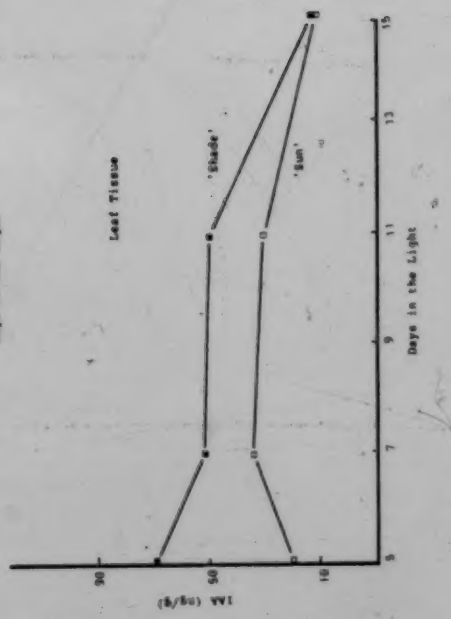
Leaves of 'shade' grown plants showed higher IAA levels for the earlier part of the growth period, days 5, 7 and 11, but by day 15 there were no apparant differences. This is shown in Figure 8. Leaves have IAA concentrations intermediate between roots and stems.

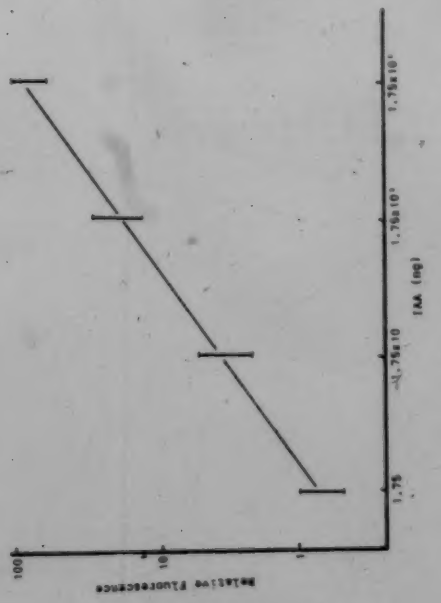
4.3.2 Bioassay

Figure 9 shows the standard curve for the Meudt-Bennett bioassay and the associated 95% confidence limits. Figures 9 and 10, respectively, shows the endogenous IAA for the first internodes, roots and leaves as determined by the Meudt-Bennet bioassay. The root tisues had a concentration in the range of 0.6 to 1.0 ng/g IAA; the first internodes of 100 to 600 ng/g. IAA and the leaves in the range of 40 to 90 ng/g IAA. The variability of both the standard curve and the sample replicates makes the separation of 'sun' and 'shade'

Figure 8. The standard curve and associated 95% confidence limits for the indole-α-pyrone physical assay and the endogenous IAA (ng/g fresh weight) in the first internode, root and leaf tissue grown in simulated sun and shade light conditions from 5 to 15 days in the light as determined by this assay.


First Internode Tissue
'Shade'
'Sun'
IAA (ng/g)
Days in the Light


Leaf Tissue
'Shade'
'Sun'
IAA (ng/g)
Days in the Light


Relative Fluorescence
IAA (ng)

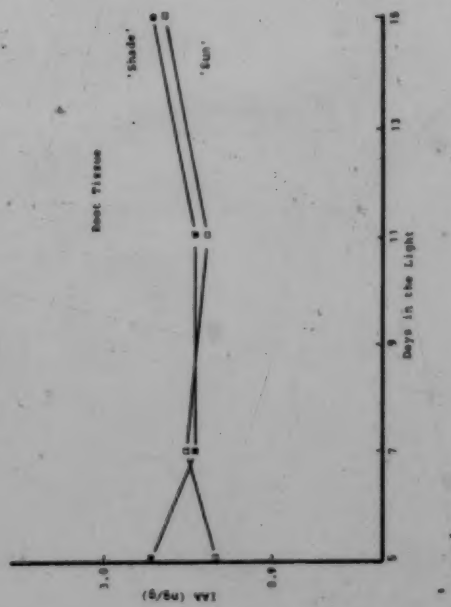
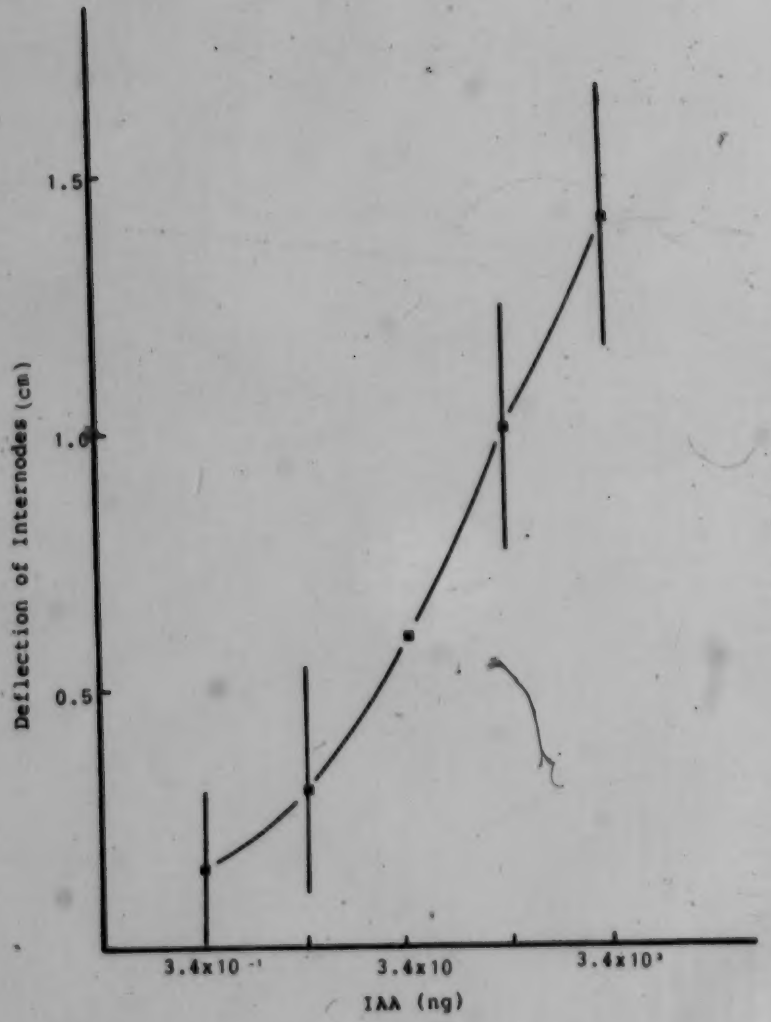

Root Tissue
'Shade'
'Sun'
IAA (ng/g)
Days in the Light

Figure 9. The standard curve and associated 95% confidence limits for the Meudt-Bennett Bioassay and the endogenous IAA in the first internode tissue grown in simulated sun and shade light from 5 to 15 days in the light as determined by the bioassay.


1.5
1.0
0.5
Deflection of Internodes (cm)
3.4x10⁻¹
3.4x10
3.4x10²
IAA (ng)

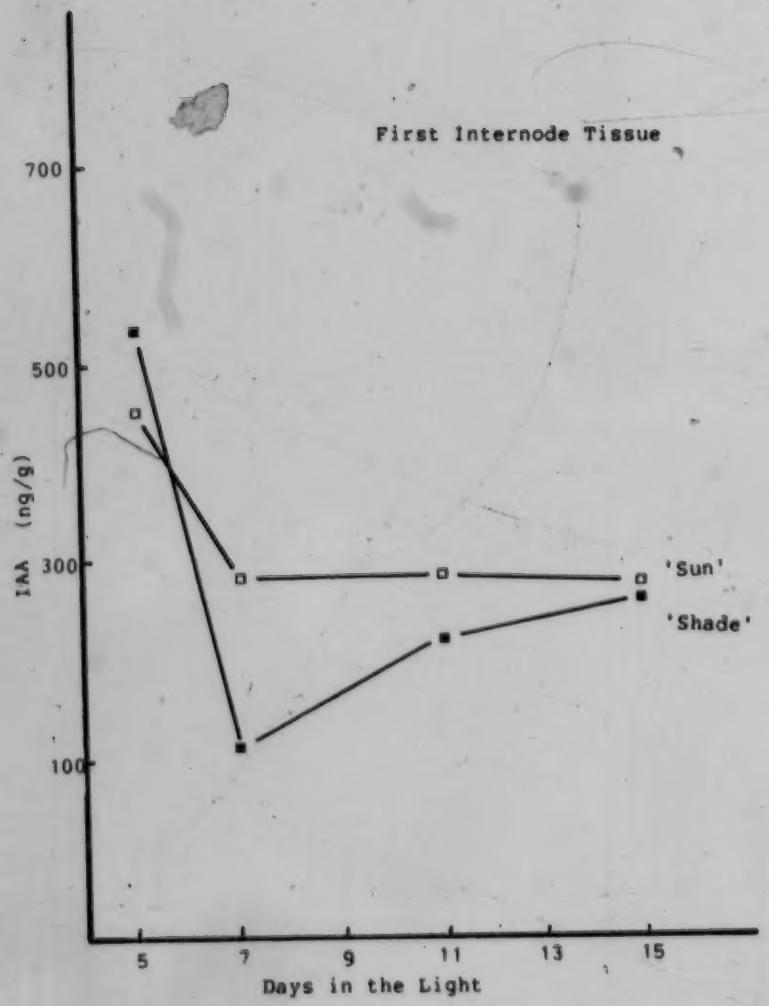
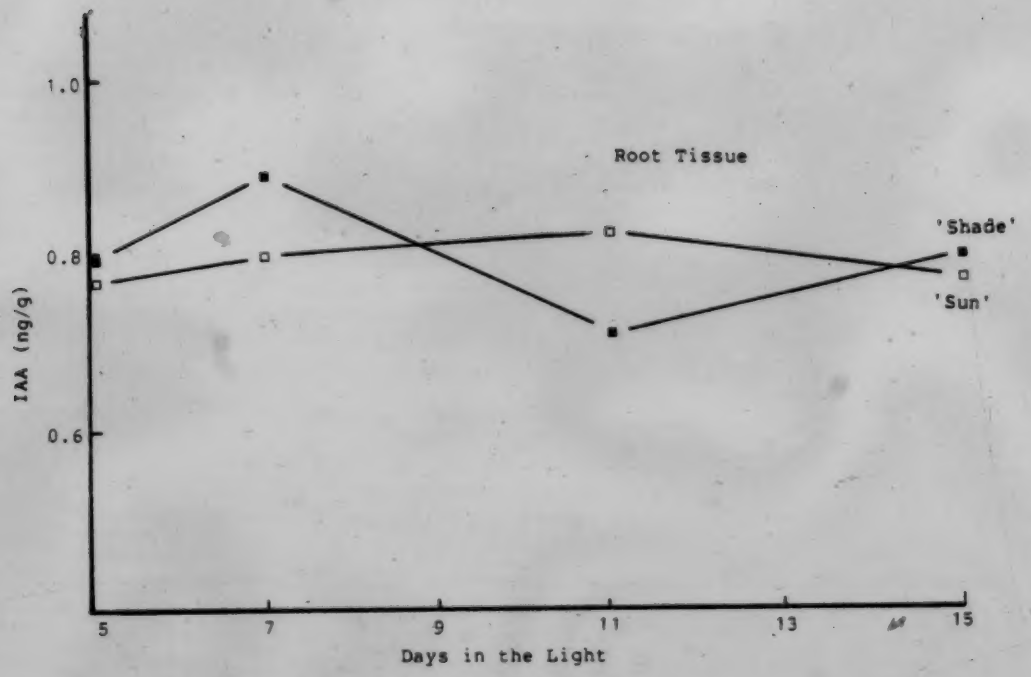
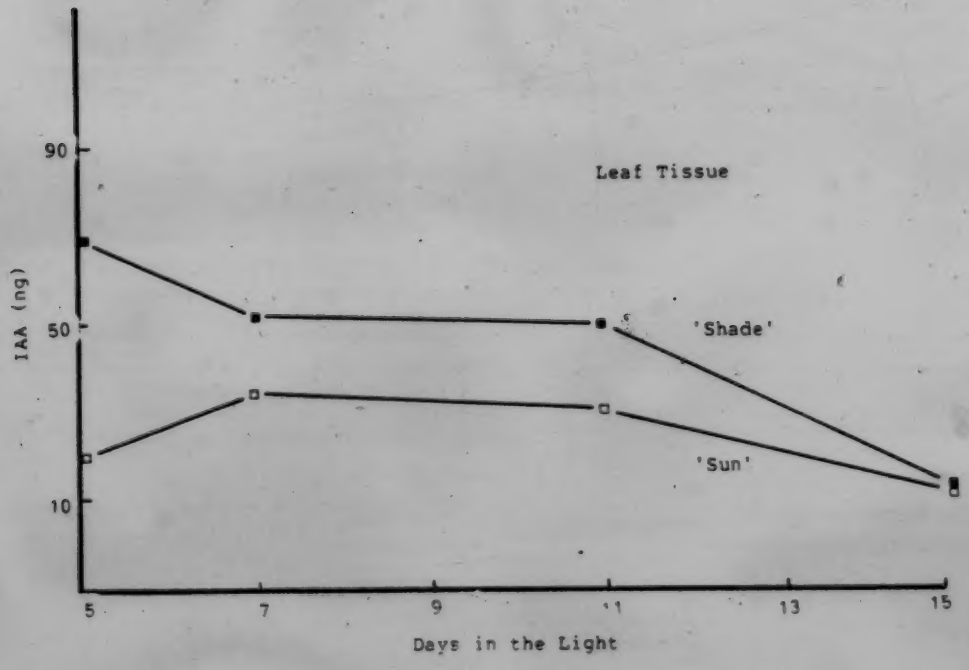

First Internode Tissue
700
500
300
100
IAA (ng/g)
'Sun'
'Shade'
5
7
9
11
13
15
Days in the Light

Figure 10. The endogenous IAA in root and leaf tissue grown in simulated sun and shade light conditions from 5 to 15 days in the light as determined by the Meudt-Bennett bioassay.


1.0
0.8
0.6
IAA (ng/g)
Root Tissue
'Shade'
'Sun'
5
7
9
11
13
15
Days in the Light


90
50
10
IAA (ng)
Leaf Tissue
'Shade'
'Sun'
5
7
9
11
13
15
Days in the Light

treatments impossible.

4.4 Discussion

"Bound" or conjugate auxins were not removed from the tissue by the extraction method. Acid hydrolyis is required for their extraction (Bandurski *et al*, 1973). This method did not separate IAA from IPyA or other indole compounds such as 4-chloroindole-3-acetic acid or 5-hydroxy indole-3-acetic acid which may have been present in the plant material and removed by the extraction procedure. In a preliminary analysis it was found that the separation of these compounds from IAA, by thin layer polyamide chromatography, reduced yield of ^{14}C labelled IAA to inadequate levels (less than 25%). These indoles have been shown to be derivatized to indole-α-pyrone by the assay procedure, however with 40% less efficiency than IAA (Hemberg and Tillberg, 1980). The concentration of these compounds in the plant tissues and the degree of interference with the actual amount of IAA measured can not be estimated.

Preliminary thin layer polyamide chromotography, followed by stainin Salkowski's Reagent, showed that the ^{14}C labelled IAA, the IAA standards and the IAA purified by the extraction and identified by physical assay had the same Rf values.

While the major problem of the physical assay was the removal of fluorescent or derivatizable compounds other than IAA; the bioassay required the removal of all other growth promotors and inhibitors. Because of this and because the bioassay depends on the variable responses of plants; bioassay are generally less specific and show more variation than physical assays.

Both the standard curve and the replicates of the bioassay showed considerably more variability than the physical assay. The bioassay did confirm the relative concentration ranges found in the physical assay but no conclusions can be drawn from bioassay results about the presence of other growth promotors or growth inhibitors that may have been present in the extract or the difference between sun and shade tissues. A bioassay used in conjunction with the indole-α-pyrone method has not been previously reported.

The levels of endogenous IAA found are in agreement with previous studies. Bandurski and Schulze (1977) investigating IAA levels in vegetative tissues and Pilet (1979) using root tissue found similar concentration ranges as were found in this study. The decrease in extractable endogenous IAA with distance from the apex has been shown previously by Scott and Briggs (1968).

Previous studies on light and light quality effects have shown that plants grown in the light have more diffusible auxin than dark grown controls (Scott and Biggs,

1968). This probably indicates a change in auxin synthesis rates or in the rates of auxin transport. Fletcher and Zalik (1964) using *Phaseolus vulgaris* treated with 8 hour periods of monochromatic light of different wavelengths found that light reduced the extractable IAA in apical portions of the plant. Plants treated with far red wavelengths had more IAA than those treated with red light.

Although the present study used multiwavelength rather than monochromatic light and equal irradiances rather than unequal quantum flux, it confirms the trend found by Fletcher and Zalik (1964). Plants grown in low zeta ratio (shade) conditions had increased concentrations of extractable IAA in the first internodes and young primary leaves and roots.

Because roots were kept darkened, the change in IAA concentrations is unlikely to be a direct effect of light on the root tissue probably the change in IAA is a reflection of the IAA translocated from the shoot tissue or another secondary effect.

The measured concentration of IAA was a function of the rates of synthesis, oxidation, transport in and out of the tissue and the synthesis and hydrolyis of IAA conjugates as discussed in section 2.1.5.1. At which point, or points, phytochrome had an affect on the concentration of IAA was not determined in the present study. However, the fact that the younger tissues, those presumed to be synthesizing most of the IAA, showed the greatest difference between the

treatments, would be a good indication that the phytochrome photoequilibrium changes rates of IAA synthesis.

5. IAA Oxidase Isozymes in Tissues of Plants Grown in Simulated Sun and Shade Light Conditions

"As plant cells age, their *in vitro* ability to destroy the plant growth hormone indoleacetic acid (IAA) increases progressively. Such IAA oxidase activity rises in young cells after pretreatment with IAA. This inducible system provides a biochemical explanation for aging, and rhythmic growth in plants".

Galston and Dalberg, 1954.

5.1 Introduction

Indole-3-acetic acid oxidase (IAA oxidase) activities have been reported to increase after red light flashes. This effect was shown to be far red reversible and therefore considered to be controlled by phytochrome (Galston and Dalberg, 1954). It has also been shown that IAA oxidase exists as multiple isozymes which may be inhibited or synthesised in response to the developmental stage of the tissue (Sharma *et al* 1979) or exogenously applied hormones (Lee, 1971 a, b, c).

This study investigates the effect of simulated sun and shade conditions on IAA oxidase isozyme band patterns. *Phaseolus vulgaris* is grown in two different light conditions which were equal in irradiance but had zeta

ratios which altered the phytochrome photoequilibrium to those found in sun and shade light conditions. The root, first internode and leaf tissues were sampled over a 15 day developmental period and the IAA oxidase isozyme bands separated using polyacrylamide gel electrophoresis.

5.2 Materials and Methods

Seedling of *Phaseolus vulgaris* L. var. Black Valentine were grown under conditons as described in section 3.2.1. After 7, 9, 11, and 15 days in the light plants were harvested and approximately 1.0 g fresh weight portions of primary leaves, roots and first internode tissues were weighed and homogenized in a ground glass homogenizer with 5 ml of Extraction Solution (Table 2). The crude homogenate was centrifuged at 1500 rpm for 40 minutes, the supernatant decanted and the pellet discarded. The supernatant was made up to 10 ml with Extraction Solution and divided into 10, 1 ml aliquots. These were frozen immediately in liquid nitrogen and stored at -20° C.

Isoymes were separated using a Bio-rad Model 1415 Horizontal analytical polyacrylamide gel electrophoresis system (Terochem, Edmonton, Alta.).

The gel plates were prepared by mixing the Casting Gel Solution (Table 3), degassing for five minutes under reduced pressure and injecting the degassed solution between a glass plate and the casting tray, using a pipette. The casting gel

MONOMER SOLUTION	
Acrylamide	24.25 g
BIS*	0.75 g
distilled water to 100 ml	
GLYCEROL SOLUTION	
glycerol	25 g
distilled water to 100 ml	
RIBOFLAVIN SOLUTION	
Riboflavin-5-Phosphate	50 mg
distilled water to 50 ml	
AMMONIUM PERSULFATE SOLUTION	
Ammonium Persulfate	100 mg
distilled water to 5 ml	
TEMED* SOLUTION	
TEMED*	0.125 ml
distilled water	9.9 ml
CASTING GEL SOLUTION	
Monomer solution	6 ml
Glycerol solution	6.0 ml
distilled water	16.5 ml
Riboflavin solution	0.15 ml
Ammonium Persulfate solution	0.15 ml
TEMED solution	0.15 ml

*BIS N, N' - Methylenebisacrylamide

*TEMED N, N, N', N' - tetrametheythenedamine

Table 2. The contents of the Casting Gel Solution used for the IAA oxidase isozyme separation.

EXTRACTION SOLUTION

TRIS*	0.06 M
PVP - 10*	8.0 g
Sucrose	10.0 g
EDTA*	0.17 g
DTT*	0.015 g
NAD*	0.005 g
NADPA*	0.005 g
Pyridoxal-5-phosphate	0.005 g
ph 6.7	

STOCK BUFFER SOLUTION

TRIS*	6.0 g
Glycine	28.8 g
distilled water to 1 liter	
ph 8.3	

STAIN BUFFER SOLUTION

1 M Na Acetate	500 ml
distilled water to 1 liter	
ph 5.0	

IAA OXIDASE STAIN

IAA*	68.4 mg
p - coumaric acid	65.6 mg
H_2O_2	0.4 ml
95% ethnol	20 ml
Fast BB blue	400 ml
Stain Buffer solution	380 ml

* DDT d-Dithiotheitol
* EDTA Ethylenediamine tetraacetic acid
* IAA Indole-3-acetic acid
* NAD nictotinamide adenine dinucleotide
* NADP nictotinamide adnine dinucleotide phosphate
* PVP polyvinyl pyrorolidone
* TRIS 2 - amino - 2 - (hydroxymethyl) - 1, 3 propanediol

Table 3. The contents of the Extraction Solution, Stock Buffer Solution, Stain Buffer Solution and IAA Oxidase Stain Solution for separation of IAA oxidase isozymes.

was allowed to polymerize for one hour under fluorescent light before the glass plate and adhering gel were removed from the casting tray.

The cooling stage of the Bio-rad equipment was wet with distilled water and the glass plate placed on it, gel upper most. Buffer chambers were filled with prechilled buffer solution at 5°C. (Table 2) and wicks, made of chromatography paper (Whatman No. 1) were wet with the buffer solution and placed between the buffers and the sides of the gel. Rectangular slots (0.5 cm x 0.25 cm) were cut into the gel 3 cm from the cathodal buffer wick and the gel removed from the slots. The slots were then filled with the thawed plant samples, and bromophenol blue was added as a marker.

A constant current of 200 volts was run for 10 minutes and then the current was increased to 400 volts for about 2 hours, until the marker dye reached the anodal buffer wick. The temperature of the gel was maintained at 5° C. thoughout the experiment by the flow of chilled water through the cooling stage. The glass plate and gel were then removed to a staining tray where they were immersed in IAA Oxidase Stain (Table 2). They were placed in the dark for 20 hours and then rinsed with distilled water. Areas containing IAA oxidase isozymes stained brown against the yellow gel.

5.3 Results

Root isozymes were designated R1, R2, . . . R4; R1 being the anodal isozyme and R4 the most cathodal. When the bands appeared within the same zone that formerly showed a single band, they were named *a* and *b* ; *a* being the more anodal isozyme. Similarily leaf isozymes were labelled L1, L2 and L3. Zymograms were drawn to indicate the thickness of bands as well as the intensity of staining, with the solid lines being more intensly stained than the dashed lines.

No discernable isozyme bands were found in first internode tissues of 'sun' or 'shade' grown plants.

The isozyme patterns of root tissue after 7, 9 11 and 15 days in the light (Figures 11 and 12) show that 'shade' light promotes the development of R2*a* and R2*b* at 15 days in the same zone in which R2 was present in 'sun' roots. R3*a* and R3*b*, after 9 and 11 days in the light appear in the same zone as R3 was present in the 'sun' roots. 'Sun' light promoted the development of R1 at an earlier stage of growth than 'shade' light.

The isozyme bands and zymograms of leaf tissue grown for 7, 9, 11 and 15 days in the light are shown in Figures 13 and 14. The development of L1 and L2 was inhibited by 'sun' light, but they appeared in 'shade' leaves; L1 at 11 days, L2 at 15 until 9 days in the light. days. The development of L3 was delayed in both light treatments

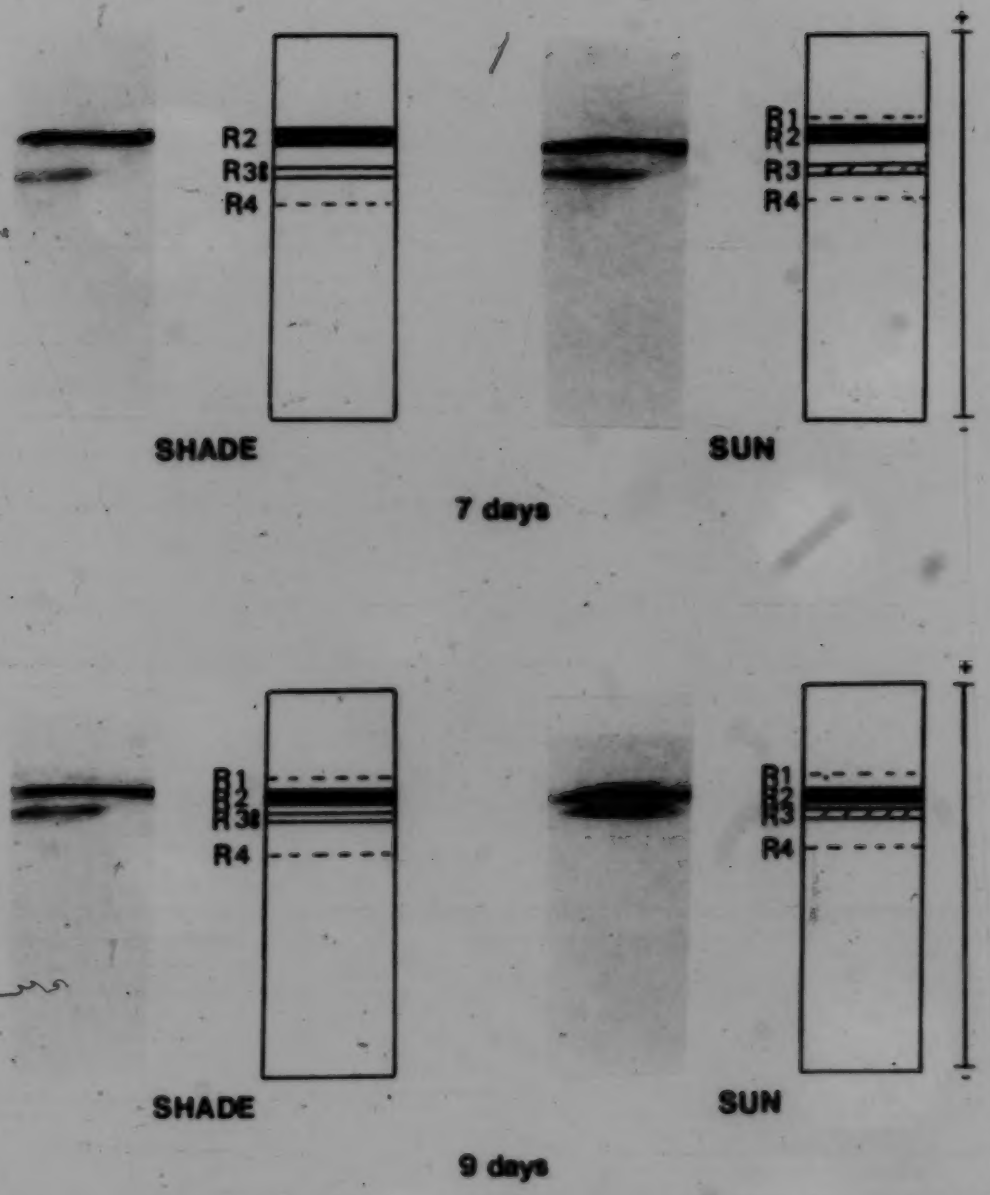


Figure 11. The IAA oxidase banding patterns and associated zymograms of root tissue grown in simulated sun and shade light at 7 and 9 days in the light.

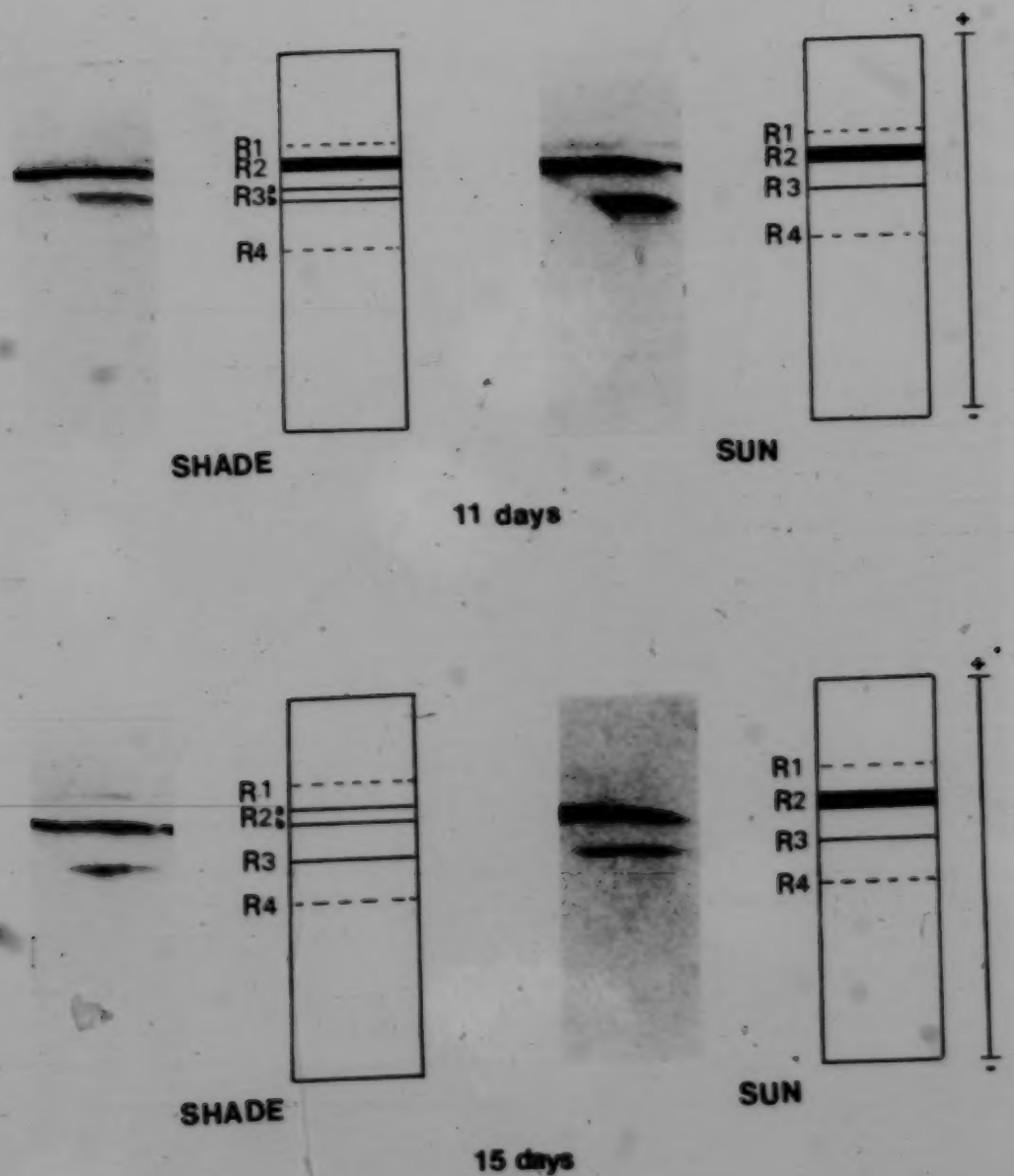


Figure 12. The IAA oxidase banding patterns and associated zymograms of root tissue grown in simulated sun and shade light at 11 and 15 days in the light.

Figure 13. The IAA oxidase isozymes in leaf tissue grown in simulated sun and shade light from 7 to 15 days in the light. The labeled zymograms follow on the subsequent page.

Leaf 79 omitted in page numbering

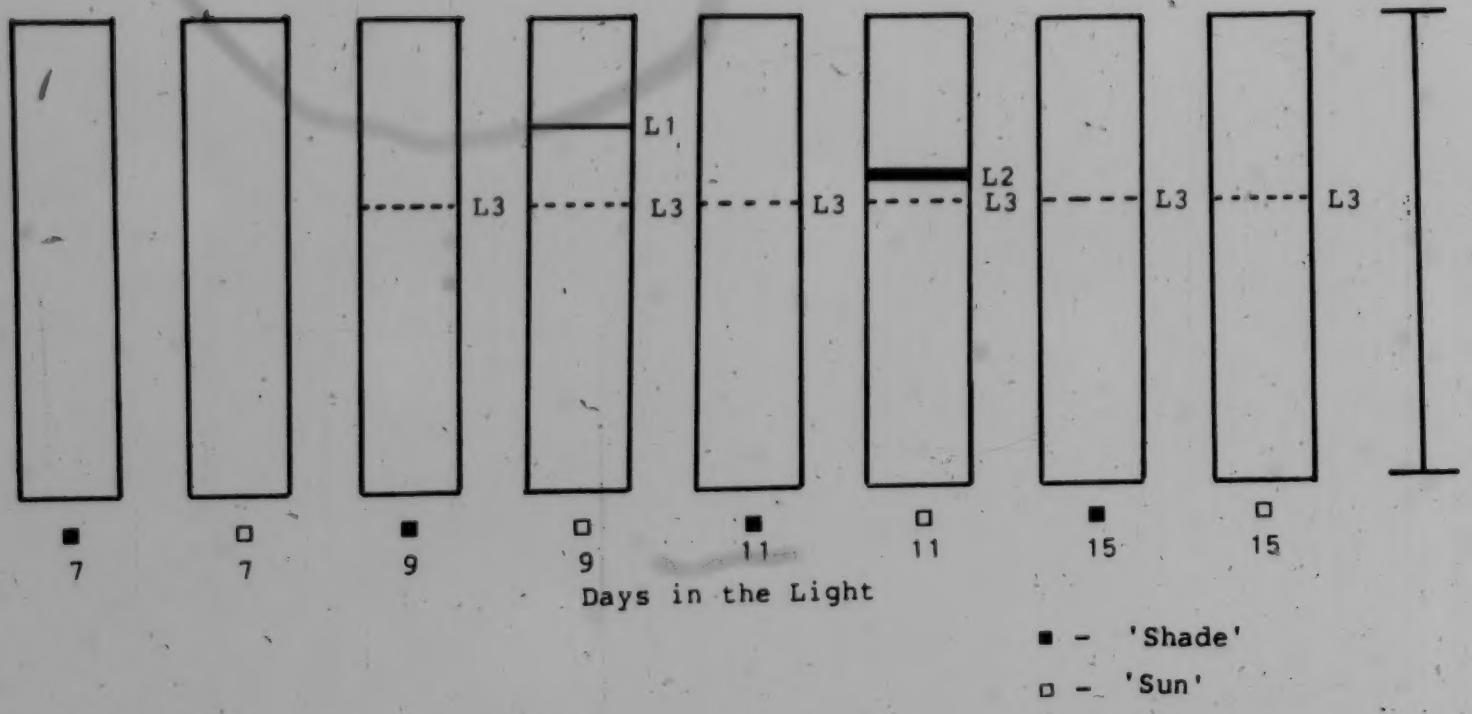


Figure 14. The zymograms of IAA oxidase isozymes in leaf tissue grown in simulated sun and shade light from 7 to 15 days in the light.

5.4 Discussion

Simulated sun and shade light conditions affected the development of IAA oxidase isozymes in both root and leaf tissue but not in first internode tissue of *Phaseolus vulgaris*. These effects changed according to the developmental stage of the plant. Similar changes during development were reported by Scandalios (1974) and Sharma *et al* (1979).

The stain used was specific for IAA oxidase, however no direct relationship between band size and IAA oxidase activity can be assumed (Gordon and Henderson, 1973). Consequently, no direct comparisons with studies on light effects on enzyme activities are made.

Rapid effects of red and far red light on enzyme activity have been shown for a large number of enzymes. The investigation of these effects has been used to determine the sites of regulation of gene expression by phytochrome. Although there is evidence to suggest that phytochrome can have a direct influence on *de novo* synthesis of some enzymes (Anstine *et al*, 1970), in this study, the mechanism of promotion of the development of isozymes by 'shade' light can not be identified.

'Sun' and 'shade' light effects on isozyme bands in leaf tissue may be due to a direct influence of phytochrome on gene expression. However, because the roots were kept darkened, it is unlikely that the altered isozyme bands in the roots were due to a direct effect of light on root

tissue. There is evidence to suggest that hormone concentrations affect the induction and repression of IAA oxidase isozymes. Lee (1971 a, b, c) using tobacco callus culture reported that increased gibberellin and IAA concentrations induced the development of rapidly migrating isozymes of IAA oxidase. The effect of IAA was dependent on the presence of kinetin in the culture medium. The inhibitors actinomycin D and cyclohexamide repressed the development of isozymes induced by IAA and gibberellin which suggests that RNA and protein synthesis is necessary for IAA to influence IAA oxidase isozymes. The enhanced development of isozymes by 'sun' and 'shade' light could, similarly, be due to a change in endogenous hormone levels as a result of translocation from other parts of the plant; or other stimuli transmitted from the shoot (de Greef *et al*, 1976).

IAA oxidase is related to peroxidase, although the nature of the relationship may vary between species. Srivastava and van Huystee (1973) working with peanut cell suspensions and Shinshi and Noguchi (1975), using cotton, found that IAA oxidase and peroxidase exhibited the same electrophoretic patterns and suggested that these were apoenzymes. This was contradicted by a report of Lu *et al* (1977) which stated that in tobacco callus culture, peroxidase isozymes did not show the same banding patterns as IAA oxidase isozymes. Because IAA oxidase and peroxidase may represent two different active sites on one molecule, the effect of phytochrome on peroxidase activity may be

relevant to IAA oxidase activities.

Peroxidase activities have been reported to follow kinetic patterns of photomodulation in maize (Sharma *et al* 1979) and photodetermination in mustard (Schopfer, 1976). Sharma *et al* (1976), using maize, showed that red light increased IAA oxidase activity in leaves but not in roots. The response was found to be age dependent and tissue dependent as was found in the present study.

Schopfer (1976) reported that, in mustard, the increase in peroxidase activity involved two sequential steps which did not overlap. The first was an inductive period during which light was effective but the response was latent. This was followed by a realization period during which red or far red light had no effect but enzyme activity increased, providing red light had previously been perceived. This indicates that in the present study, the developmental period during which the change in isozymes occurs is not necessarily the same as the period of the initial light influence.

The finding of the present study, that changes in 'sun' and 'shade' light were associated with changes in IAA oxidase isozyme bands, implies that the inhibition and development of IAA oxidase isozymes may be one of the controlling factors in photomorphogenesis in *Phaseolus vulgaris*.

6. General Discussion

"The idea is like grass; It craves light, likes crowds, thrives on crossbreeding, grows better for being stepped on."

U. L. LeGuin, 1974.

The present study has shown that long term 'sun' and 'shade' conditions influence the gross morphology, endogenous IAA concentrations and the pattern of IAA oxidase isozyme bands of *Phaseolus vulgaris*.

These studies raise questions on the relationship between IAA and IAA oxidase isozymes, and between IAA and plant morphology.

6.1 IAA - IAA Oxidase Relationships

Stem tissue and young leaf tissue, which had the highest levels of IAA in both 'sun' and 'shade' conditions, had no discernable IAA oxidase isozyme bands present. The highest IAA levels in the first internode occured when they were immediately basipetal to the apices, a major site of IAA synthesis. The high levels of IAA in the leaves might also have been due to high rates of synthesis of IAA as they have also have been reported to be primary sites of synthesis. After 9 days in the light, when most primary leaf

expansion had taken place IAA oxidase isozyme bands were discernable and there was a corresponding decrease in IAA concentration.

Roots showed the greatest IAA oxidase isozyme band number and intensity as well as the lowest IAA concentrations. Only the young roots showed different IAA levels in the 'sun' and 'shade'. Although there was no apparent relationship between band numbers, light treatments and IAA levels and IAA oxidase activity was not directly assessed; it can be stated that tissues with low concentrations of IAA potentially had the greatest IAA oxidase activity.

The lack of an absolute association between the number and intensity of IAA oxidase isozyme bands and IAA concentrations is not suprising in the light of the number of possible mechanisms for the control of IAA concentrations. Some of these have been shown previously be influenced by phytochrome; phenolic cofactors and inhibitors, (Bottomely *et al* 1975), IAA oxidase activity (Galston and Dalberg, 1954) and the transport of IAA (Yamaki and Fujii, 1968). Changes in the phytochrome photoequilibrium could be affecting IAA levels though changes in one or more of these processes. Other mechanisms have also been show to affect IAA concentration but as yet have not been related to phytochrome, including IAA conjugation and IAA protectors.

Multiple points of control of IAA concentrations by phytochrome are certainly possible. Since IAA is such an important regulator of growth and development, it is likely that a regulatory and feedback system is operative. Similar multiple regulation systems have been suggested for enzymes by Fosch *et al*, (1977).

6.2 IAA-Morphology Interactions

There was generally a strong relationship between the concentrations of endogenous IAA and the rate of growth in *Phaseolus vulgaris*. Higher concentrations occurred in young tissues which have the highest growth rates, and in the 'shade' plants which had higher growth rates than the 'sun' plants.

The decrease in endogenous IAA concentrations with time in the leaf tissue was associated with a decrease in leaf expansion rates. In a previous study, Hoddinott and Hall (In Press) found that primary leaves of *Phaseolus vulgaris* in 'shade' conditions had, after approximately 11 days in the light, a greater rate of expansion than in 'sun' conditions. There were corresponding differences in IAA concentrations in 'shade' and 'sun' leaves. When leaves had stopped expanding and began to show signs of senescence at about 14 days (decreases and variablity in chlorophyll content), decreases were seen in endogenous IAA in both 'sun' and 'shade' conditions. This is consistent with the observations

of Wheeler (1968) that in *Phaseolus vulgaris* the auxin levels increased to a maximum during rapid leaf expansion and were reduced to low levels as the leaves matured. Exogenous IAA, applied to leaves, induced the development of main veins but not of lamina (Goodwin, 1978). Leaves with large vacuolated parenchymatous tissue and vascular bundles but little mesophyll have been reported in response to IAA application in *Phaseolus vulgaris* (Goodwin, 1978) but this may be due to the abnormally high endogenous concentrations this causes.

The roots of 'shade' plants had higher growth rates, in terms of increases in dry weight, than the 'sun' grown roots, but they showed higher levels of IAA only in the 5 day old root. Goeschl (1978) in reviewing interactions of hormones, growth and differentiation found no clear evidence for a correlation between endogenous auxin, cytokinin or gibberellin distributions and growth rates of roots, However, auxin applied to *Zea mays* root bases at concentations of 10^{-7} M has been shown to stimulate root growth (Goodwin, 1978).

First internode tissue showed that the concentrations of IAA were higher in 'shade' grown plants which have the highest relative growth rates. Fast elongation rates have been shown previously, to correspond with high IAA concentrations. In Norway spruce, the highest auxin activity occurred just prior to the time of maximum stem elongation (Goodwin, 1978). However, applied auxins have been reported

to have little or no promotive effect on stem elongation in a number of species, including *Phaseolus vulgaris* (Goodwin, 1978). However auxin transport inhibitors have an inhibitory effect on stem elongation (Schneider, 1972). This could indicate that the application of auxin, in combination with the already high levels of IAA in the stem, become inhibitory to growth.

There are of course many other factors shown to be affected by phyto which could have an influence on plant morphology. As discussed in the Literature Review (Section 2.1.5) all plant hormones, both promotors and inhibitors have been shown to be effected by phytochrome in some species. Although most of the studies on hormones and phytochrome levels have been carried out using monochromatic light and different plant species and tissue and the results are not entirely comparable; they indicate that there is a strong possibility that IAA was not the only hormone affected by changes in the phytochrome photoequilibrium.

It has also been demonstrated that hormones interact, that is the changes in the concentrations of one hormone will affect the concentrations Application of gibberellins to plants results in increases in endogenous auxins (Schneider *et al*, 1978). Cytokinins also have a promotive affect on IAA levels and may be important in maintaning endogenous IAA levels. Increases in ethylene concentrations reduce IAA levels (Schneider *et al*, 1978). Combinations of hormones may show different effects than those obtained for

a single hormone. (Schneider and Wightman, 1978)

These complex interrelationships make it difficult for a causal relationship between IAA, IAA oxidase and morphology to be definitively established. However, it is clear that there is a correlation between the phytochrome mediated responses to shade conditions discussed herein.

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